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Performance Assessment of Bi-Directional Knotless Tissue-Closure Device in Juvenile Chinook Salmon Surgically Implanted with Acoustic Transmitters, 2010

Final Report

CM Woodley
AJ Bryson
SM Carpenter
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September 2012

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Pacific Northwest National Laboratory
Marine Sciences Laboratory
Sequim, Washington 98382

Executive Summary

The purpose of this two-week study, conducted by Pacific Northwest National Laboratory for the U.S. Army Corps of Engineers, was to assess the performance of a bi-directional knotless tissue-closure device and to determine the optimal suture pattern and needle circumference needed for in river monitoring and research of juvenile salmonids.

In summer 2010, run-of-the-river (ROR) juvenile subyearling Chinook (SYC) salmon were implanted with a Juvenile Salmon Acoustic Telemetry System (JSATS) acoustic transmitter (AT) and passive integrated transponder (PIT). The incisions were closed with four separate treatments using a bi-directional knotless tissue-closure device[1]. The four treatments consisted of three different suture patterns and two needle sizes. Fish were examined on 7 and 14 days post-implantation for suture loss, incision openness and redness, and ulceration in the area of the incision. Fish were continuously monitored for moribund behavior or mortalities and tag loss. On day 14, all fish were euthanized and necropsies were conducted to confirm the presence of each AT, PIT, and suture material.

Mortalities, AT loss, incision openness, functional suture (including presence and tension across wound), ulceration, redness, tag bulging, and tissue fibrosis were examined and the frequency of occurrence for each of these factors was incorporated by treatment group to determine an average performance ranking (1 to 4). Although the results may be confounded by the small sample size and thus low statistical power on most tests the performance index indicated that the Wide "N" Knot 12 treatment group overall performed *better* than the other treatment groups, although it was not consistently superior. The mortality rate in this study was relatively low (11%) and no PITs were dropped; however, the Wide "N" and Wide "N" Knot 18 treatment groups had an AT loss of 33%. The high AT loss may be attributed to low suture functionality.

Although the Wide "N" Knot 12 had the best overall performance, all treatments had issues with suture functionality. By day 14, the 6 Point and Wide "N" treatment groups had no functional sutures, while the Wide "N" Knot 12 and Wide "N" Knot 18 had 33 to 66% functional sutures. Fish that had functional sutures appeared to have increased redness and ulceration, possibly due to inefficient anchoring of the barbs. The rigid composition of the suture may have contributed to the sutures losing suture pattern and working their way out of the tissue. We are not recommending the tested suture and suture patterns be used on juvenile SYC. The retention and rigidity appear to be more likely suitable for large adult fish and/or fish with large scales. A more flexible suture material, different barb geometry, or different number of barbs per suture may be required for use with juvenile salmonids.

[1] (Monoderm™, Quill, Angiotech Pharmaceuticals, Vancouver, BC)

Acknowledgments

The authors acknowledge the support and oversight of M. Brad Eppard, the contracting officer's technical representative for the U.S. Army Corps of Engineers. In addition, we thank Pacific States Marine Fisheries Commission employees Greg Kovalchuk, Dean Ballinger, and support scientists at John Day and Bonneville dams for their technical support and advice during the project. We also acknowledge the large project teams of the Lower Columbia River Acoustic-Tag Investigations of Dam Passage Survival and Associated Metrics study (both 2010 and 2011), without whose support this concurrent study could not have been accomplished. Lastly, we would like to thank our thorough reviewer and editor, Joanne Duncan and Susan Ennor, respectively, for their attention to details and excellent comments. Thank you.

Animal facilities were certified by the Association for Assessment and Accreditation of Laboratory Animal Care, animals were handled in accordance with federal guidelines for the care and use of laboratory animals, and protocols were approved by the Institutional Animal Care and Use Committee, Battelle–Pacific Northwest Division. Reference to trade names does not imply endorsement by the U.S. Government.

Acronyms and Abbreviations

°C	degree(s) Celsius or Centigrade
AT	acoustic transmitter
ANOVA	analysis of variance
BiOp	Biological Opinion
BON	Bonneville Dam
F	F-test statistic
FET	Fisher's Exact Test
FCRPS	Federal Columbia River Power System
FL	fork length
g	gram(s)
gal	gallon(s)
h	hour(s)
JDA	John Day Dam
JSATS	Juvenile Salmon Acoustic Telemetry System
L	liter(s)
mg/L	milligram(s) per liter
mm	millimeter(s)
mm^2	millimeters squared
MS-222	tricaine methanesulfonate
N	replicates
$NaHCO_3$	sodium bicarbonate
P	p-value; probability of test statistic
PIT	passive integrated transponder
PNNL	Pacific Northwest National Laboratory
rkm	river kilometer(s)
ROR	run-of-the-river
SD	standard deviation
SMF	Smolt Monitoring Facility
SYC	subyearling Chinook salmon
USACE	U.S. Army Corps of Engineers
WW	wet weight

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1.0 Introduction

In 2010, researchers at Pacific Northwest National Laboratory (PNNL) and the University of Washington (UW) conducted a compliance monitoring study—the Lower Columbia River Acoustic Transmitter Investigations of Dam Passage Survival and Associated Metrics 2010 (Carlson et al. in preparation)—for the U.S. Army Corps of Engineers (USACE), Portland District. The purpose of the compliance study was to evaluate juvenile Chinook salmon (Oncorhynchus tshawytscha) and steelhead (O. mykiss) passage routes and survival through the lower three Columbia River hydroelectric facilities as stipulated by the 2008 Federal Columbia River Power System (FCRPS) Biological Opinion (BiOp; NOAA Fisheries 2008) and the Columbia Basin Fish Accords (Fish Accords; 3 Treaty Tribes and Action Agencies 2008).

Current acoustic telemetry studies require invasive surgical techniques for transmitter implantation. Ongoing efforts have focused on reducing this invasiveness to address telemetry and survival model assumptions. Prior research has indicated that suture material and technique can be destructive to fish tissue, externally and internally (Wagner et al. 2000; Deters et al. 2010). Recovery from surgery, including the up-regulated immune system response to tissue damage, may result in the "tagged" fish ("tagged" herein referring to a fish that underwent surgical intracoelomic implantation) not being equivalent to or representative of the population of interest due to its altered physical and physiological state. Researchers at PNNL have been conducting research on suturing techniques, suture materials, and tag burdens in an effort to reduce the unwanted effects of tags and tagging procedures (Brown et al. 2010; Deters et al. 2010; Carter et al. 2011; Cooke et al. 2011). In 2009, we examined several novel incision closure techniques and found that the novel approaches did not perform better than two discontinuous sutures (MonocrylTM monofilament) with a 2 x 2 x 2 x 2 knot treatment for tissue trauma and tag retention in 17°C water. However, the novel approaches, at both 12 and 17°C exposures, were faster to execute, which resulted in reduced time anesthetized (Woodley et al. 2011).

In 2010, this approach was used to refine the knotless (barbed) suture and suture patterns used in river monitoring and research programs. On August 3, 2010, outmigrating subyearling run-of-the-river (ROR) Chinook salmon were implanted with Juvenile Salmon Acoustic Telemetry Systems (JSATS) micro-acoustic transmitters (ATs; each 12 mm long × 5 mm wide × 4 mm high, 0.43 g in air), and passive integrated transponders (PITs), using a bi-directional knotless tissue-closure device (MonodermTM, QuillTM, Angiotech Pharmaceuticals, Vancouver, BC) to close the incision. In this study, the effects of 3 suture patterns and 2 needle sizes on incision healing were examined over 14 days. The study was conducted at the Bonneville Dam (BON) Smolt Monitoring Facility (SMF). Test fish were examined on post-surgical days 7 and 14, euthanized on day 14, and necropsied for internal assessment of suture and tag effects.

1.1 Background

Telemetry applications for fish range from monitoring fine spatial movements and habitat preferences, to monitoring large-scale migratory patterns and passage survival (Skalski 1998; Scruton et al. 2007). In the Columbia and Snake rivers, scientists have identified acoustic telemetry as an essential technology for observing behavior and estimating survival of juvenile salmonids passing through the main-stem FCRPS and associated side channels (Faber et al. 2001; McComas et al. 2005; Ploskey et al. 2008; Clemens et al. 2009). Hydroelectric dams provide various routes of passage where mortality becomes pathway-specific depending on the physical properties of the technical installation (i.e., route through turbines, spillways, bypass structures, etc.; Coutant and Whitney 2000; Muir et al. 2001; Skalski et al. 2002; Weiland et al. 2009). In addition, impoundments and passage facilities may delay juvenile salmonid outmigration, conceivably increasing their exposure to predators and contributing to disease. Because of the direct and indirect threats to salmonids caused by impoundments, telemetry and survival models are used to monitor passage. Both telemetry and survival models, though, assume tagged animals (whether external or internally implanted devices are used) to be representative of the population under evaluation, and not to exhibit behavioral, physiological, or survival differences when compared to the untagged populations.

Acoustic transmitters, when used in fish survival studies, are often surgically implanted into the coelomic cavity of the fish. Surgical implantation is a well-established method for studying fish movements and survival through structures, but this technique has disadvantages (Bridger and Booth 2003; Bauer and Loupal 2007; Chittenden et al. 2009; Frost et al. 2010; Gheorghiu et al. 2010). The tag or the surgical procedure may potentially alter the behavior, growth, or survival of the fish (LaCroix et al. 2004; Chittenden et al. 2009; Stephenson et al. 2010). In addition, transmitter loss (or shedding) can occur due to foreign body rejection response (often referred to as "tag expulsion"), poor tissue apposition causing the transmitter to exit the incision (CBSPSC 2011), or application of external mechanical forces, such as pressure (Stephenson et al. 2010). If transmitters are expelled, a false mortality rate will be estimated; or if the tagging process decreases fish fitness or contributes to mortality, fish are no longer representative of the population under investigation. Poor surgical procedures, including prolonged exposure to anesthetic (Congleton 2006; Rombough 2007), "unsanitary" conditions[1] (Harms 2005; Leaper 2010), poor surgical techniques resulting in tissue trauma or incision gaping (Fortenot and Neiffer 2004; Harms 2005), or inefficient post-implantation recovery time (Harms 2005) can result in altered behavior, growth, and/or survival.

After inserting a telemetry device (e.g., an AT) into the coelomic cavity of a fish, the incision must be closed to prevent transmitter expulsion and pathogen entry, minimize changes in physiological state due to osmotic stress, and support tissue healing (Jepsen et al. 2002; Mulcahy 2003). Based on prior research, synthetic monofilaments may elicit less tissue inflammation and promote more rapid incision healing than silk sutures (Cooke et al. 2003; Jepsen et al. 2008; Deters et al. 2009). For example, rainbow trout (*O. mykiss*) experienced less tissue inflammation from synthetic monofilament than from braided silk sutures (Wagner et al. 2000). Similarly, Deters et al. (2010) found that wound inflammation and ulceration were generally lower with the use of synthetic monofilament compared to braided sutures in yearling juvenile Chinook (held at 12 and 17°C). As a result of studies like these, the Columbia Basin Surgical Protocol Steering Committee has recommended the use of absorbable synthetic monofilament suture material tied in a simple interrupted suture pattern for closing surgical incisions in fish (CBSPSC 2011).

Wound closure in fish is a process involving several actions to produce a functional suture. A functional suture is defined as a suture in the fish that is knotted, has appropriate tension across the wound, and does not tear through the body wall of the fish (modified from Deters et al. 2009). Non-functional sutures result in slow tissue healing, osmotic stress, tissue damage, or possible premature

[1] Aseptic or sterile surgeries are not feasible because a fish's mucous coat (barrier) is its first line of defense and should not be compromised. Surgical scrubs and disinfectants used on terrestrial animals could harm or degrade the mucous barrier and/or damage the skin and gills of fish.

mortality (Fortenot and Neiffer 2004; Harms 2005; Greenburg and Clark 2009). Ideally, the suture material should be placed in the tissue so that the incision margins are and remain approximated, thereby minimizing open spaces and aiding in healing (Lin et al. 1996; Wagner et al. 2000; Bridger and Boothe 2003; Fortenot and Neiffer 2004). Excessive suture tension on tissue can cause ischemic areas that reduce or slow revascularization; increase stretching, tearing, and necrosis; and ultimately slow healing. Improperly tied knots can become untied, thereby releasing wound margins, slowing healing, and allowing transmitter loss. Large knots can be a point source for tissue irritation due to the concentrated amount of foreign material making up the knot (van Rijssel et al. 1989). Functional sutures and practices to reduce tissue damage are needed to ensure the retention of intracoelomic transmitters, and reduction of any behavioral or physiological differences between tagged fish and run of the river populations.

Currently, a novel bi-directional knotless tissue-closure device (Monoderm™, Quill, Angiotech Pharmaceuticals, Vancouver, BC) has been shown to streamline wound closure and decrease healing time. Knotless tissue-closure devices are easy to handle, reduce instrument handling and surgical time, enable the use of continuous stitching rather than interrupted sutures and knots, and most importantly provide uniformly distributed tension across the wound rather than at specific sites (Sadick et al. 1994; Shermak et al. 2009). Similar to synthetic absorbable monofilament, Monoderm™ is an absorbable monofilament in which the copolymer degrades in vivo over time. Degradation occurs by hydrolysis of the ester links in the polymer backbone, until dissolution and full absorption occurs (Angiotech 2011). Quill™ tissue-closure devices are based on the reconstruction of a traditional suture material where the suture has tissue retainers (barbs) arranged around the shaft that protrude at ~45º from the main suture shaft (Figure 1). Tissue retainers allow the suture to be pulled through the tissue, and then anchor itself, much like a porcupine quill or stingray barb, eliminating the need for a knot. Once anchored, the barbs distribute the suture tension across a larger area minimizing ischemic pressure points. The knotless design eliminates the potential for unraveling, and reduces the amount of foreign material against the tissue, which can cause irritation and allow fungal and bacterial growth.

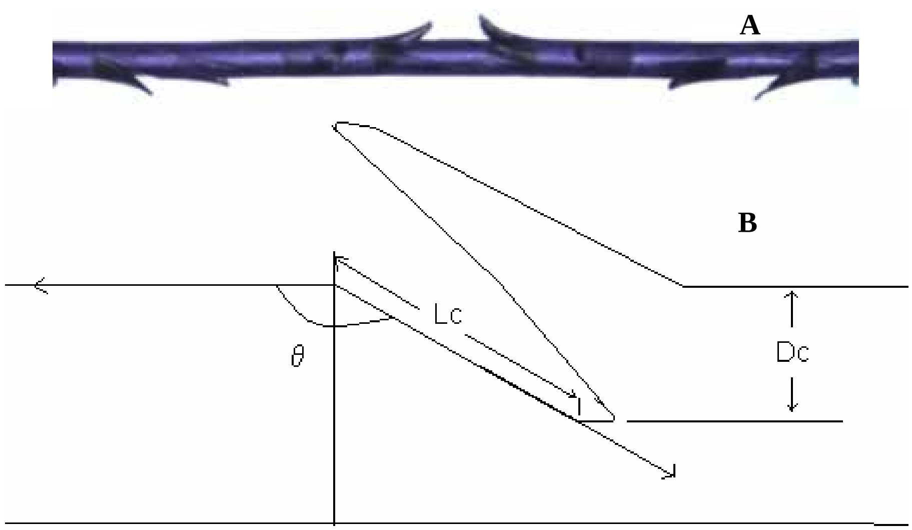

Figure 1. Knotless Suture Geometry. A) Knotless suture region where barbs transition from one direction to the other (accessed Angiotech March 15, 2011; http://www.angioedupro.com/Quill/index.php?ID=Photos). B) Individual barbs compared to the main suture shaft (Leung et al. 2003).

1.2 Purpose and Scope

The objective of this study was to assess the performance of the bi-directional knotless tissue-closure device for incision closure and wound healing in juvenile salmon. ROR juvenile yearling Chinook salmon were implanted with a JSATS AT and PIT, and the incisions were closed with separate treatments consisting of three suture patterns and two needle sizes. The wounds and suture performance were examined on 7 and 14 days post-implantation for suture loss, incision openness and redness, and ulceration in the area of the incision. The fish were continuously monitored for moribund behavior or mortalities and/or tag loss. On the 14th day, the fish were euthanized and necropsies were conducted to confirm the presence of each AT, PIT, and suture material.

Seven questions were addressed in this experiment:

1. Does one suture pattern and its associated needle type have a greater mortality rate as measured by number of fish deaths per treatment?
2. Does one suture pattern and its associated needle type yield a higher AT or PIT retention as measured by dropped ATs or dropped PITs?
3. Does one suture pattern and its associated needle type have a greater potential for tag loss and lower potential for incision healing as measured by incision openness?
4. Is one suture pattern and associated needle type more functional than the others based on the number of sutures that can be identified as functioning?
5. Does one suture pattern and its associated needle type have a greater amount of tissue trauma (ulceration and redness)?
6. Does biocompatibility (presence of fibrous tissue or tag bulge) vary with fish size or suture pattern and its associated needle type?
7. Is fish size a confounding variable?

1.3 Report Contents and Organization

The ensuing sections of this report describe the study methods and materials (Section 2.0) and results (Section 3.0), and discuss the associated findings (Section 4.0). The results of this report complement those of the compliance monitoring study conducted by researchers at PNNL and UW for the USACE. References for sources cited in the text are listed in Section 5.0.

2.0 Methods and Materials

This study, conducted during 14 days in summer 2010, involved fish acquisition, surgical implantation of ATs and PITs, and examination of responses to implantation as described below.

2.1 Fish Acquisition

On July 19, 2010, ROR subyearling Chinook salmon (SYC; N = 27) were collected at the John Day Dam (JDA) SMF (rkm 349), by PNNL and JDA SMF staff. Fish were held for 24 h and then transported to the BON SMF. Fish were placed in 80-gal (302.8 L) tanks supplied with flow-through river water for 24 h. Fish were fed Biodiet pellets (Bio-Oregon, Inc., Longview, Washington) daily at a rate of 1.1% of their body weight. Fish were not fed 24 h prior to or 6 h after surgery or weekly exams. All fish were allowed to acclimate for 24 h prior to the surgical process (see Section 2.3, Surgical Procedure).

Subyearling Chinook salmon were observed several times daily to determine if there were injuries, abnormal behavior, or mortalities. Tanks were siphoned daily to remove fecal matter and debris and to recover any ATs or PITs that may have been shed. Each tank outflow was fitted with a net bag to prevent shed tags from being lost.

2.2 Suture Patterns Mechanics

Fish were assigned randomly to one of five treatments as follows (Table 1, Figure 2):

- 6-Point Continuous Suture treatment (herein referred to as “6-Point”). This pattern had smaller angles across the incision and more insertion points than other treatments. The first point of insertion was in the middle of the incision, pulling the suture through opposing sides and ensuring the barbs were anchored in both directions. The 6-Point treatment used a 3/8 circle needle with a 12-mm circumference.
- Wide “N” Continuous Suture treatment (herein referred to as Wide “N”). This pattern had wider angles across the incision and fewer insertion points than the 6-Point treatment. The first point of insertion was in the middle of the incision pulling the suture through the opposing sides and ensuring barbs were anchored in both directions. The Wide “N” treatment used a 3/8 circle needle with a 12-mm circumference.

Table 1. Needle Configuration, Patterns, and Sample Sizes of SYC in Each Treatment. All needles were 3/8 circle diamond point.

Treatment	Needle Circumference	Knots Used	Number of Insertion and Exit Sites	N
6-Point	12 mm	0	3	6
Wide “N”	12 mm	0	2	6
Wide “N” Knot 12	12 mm	1	2	6
Wide “N” Knot 18	18 mm	1	2	3
Control	NA	0	0	6

NA = Not applicable

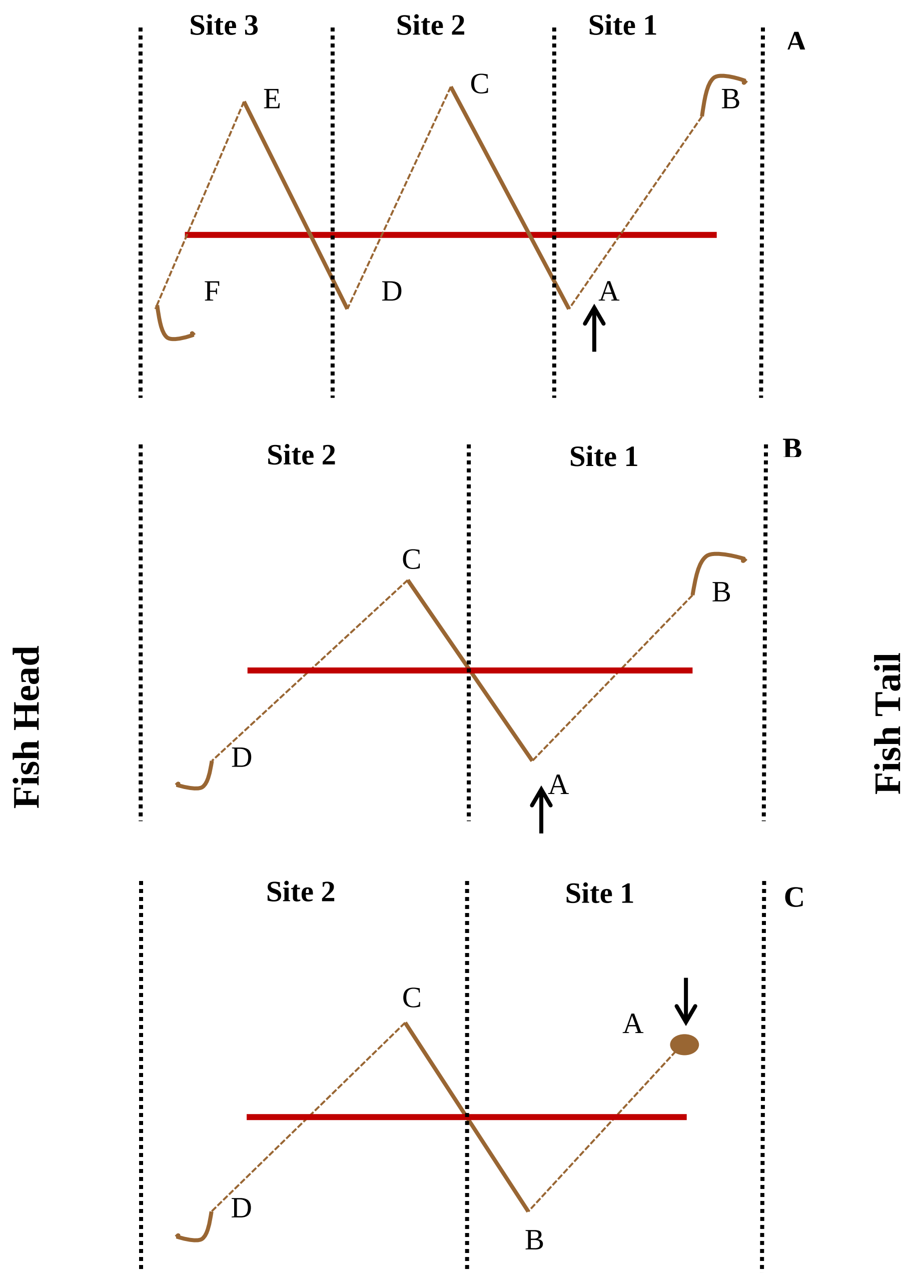

Figure 2. Schematic of the Three Suture Patterns. A) 6-Point, B) Wide "N", and C) Wide "N" Knot (both 12- and 18-mm needle sizes). Solid red lines represent incisions. Brown dashed lines represent internal suture areas. Brown solid lines represent external suture areas. Brown curved lines represent the needle. The brown solid dot is the knot tied for the Wide "N" Knot treatment (both 12 and 18 mm). Black dotted lines section the incision into sites for description purposes (site 1, site 2, or site 3; further described in Section 2.4). The 6-Point pattern has three sites, and the Wide "N" and Wide "N" Knot (both 12 and 18 mm) have 2 sites. The arrows indicate the point of first insertion. Letters represent entry/exit points of the suture (further described below).

- Wide “N” Knot Suture pattern treatment (herein referred to as Wide “N” Knot 12 or Wide “N” Knot 18, depending on needle circumference). This treatment pattern had the same angles across the wound as the Wide “N” treatment. This technique used a small knot at the end of the suture using only half of a suture. Barbs gripped in one direction, opposite the knot. Single square knots were used and placed on the suture prior to use. This technique is faster than placing a knot using a traditional suture and eliminates tissue tearing due to knot tension. The Wide “N” Knot 12 treatment was performed using a 3/8 circle needle with a 12-mm circumference, while the Wide “N” Knot 18 treatment was performed using a 3/8 circle needle with an 18-mm circumference.
- Control. These fish underwent the same handling procedure as treatment fish but were not surgically implanted. These fish were used to gauge mortality rates between treatments.

Depending on the suture pattern, there are several entry and exit points. The 6-Point pattern (Figure 2A) had two entry (points C, E) and four exit (points A, B, D, F) points. The needle entered through the incision, exiting at point A until the middle point of the barbed suture was halfway through the skin. Next, the surgeon used the internal portion of the suture to exit at point B, cutting the suture 3 mm from the entry point (i.e., leaving a 3-mm tail). The suture remaining outside of exit point A extended across the wound and entered the tissue at entry point C. The needle passed into the body cavity at point C and extended across the wound exiting at point D, before extending across the wound at point E and exiting at point F. The excess suture at point F was cut leaving a 3-mm tail (Figure 3A).

The Wide “N” pattern (Figure 2B) has four entry and exit points with wider suture angles across the wound than those of the 6-Point treatment. The needle entered through the incision, exiting the skin at point A until the middle point of the barbed suture was halfway through the skin. Next, the surgeon used the internal piece of suture to exit at point B, and the remaining suture was cut 3 mm from the entry point, leaving a 3-mm tail. The remaining suture outside of exit point A extended across the wound and entered the tissue at entry point C. The needle was passed back into the body cavity at point C and extended across the wound at point D. The excess suture at point D was cut leaving a 3-mm tail (Figure 3B).

The Wide “N” knot pattern (for both 12 and 18 mm sizes; Figure 2C) used one segment of the suture with a knot tied at the end, denoted by the circle at point A. The needle passed through the body wall into the cavity at point A, exiting at point B, and the suture was pulled until the knot met the fish scales at point A. The needle was passed back into the body cavity at point C and extended across the wound, exiting at point D. Excess suture was cut at point D.

2.3 Surgical Procedures

Surgeries were performed on ROR SYC on August 3, 2010. One surgeon performed all surgeries. During surgery, the average water temperature was 19.8°C (± 2°C). Fish were anesthetized and handled similarly regardless of treatment. A buffered anesthesia ($NaHCO_3$; 80 mg/L) was prepared using aerated river water and tricaine methanesulfonate (MS-222; 80 mg/L). Prior to surgery, fish were anesthetized in buckets until loss of equilibrium was observed (Stage 4; Summerfelt and Smith 1990). Anesthetized fish were immediately weighed, measured, and both flanks photographed. Water temperature was monitored and new water was acquired if the temperature varied more than 2°C from the initial temperature. Fish were randomly assigned to one of five treatment groups: 6-Point, Wide "N", Wide "N" Knot 12, Wide "N" Knot 18, or Control. All treatment groups underwent surgical implantation, while the Control fish bypassed the surgery stations, and were placed into 5-gal perforated recovery buckets (five fish per bucket), aerated with river water, and were monitored during recovery from anesthesia.

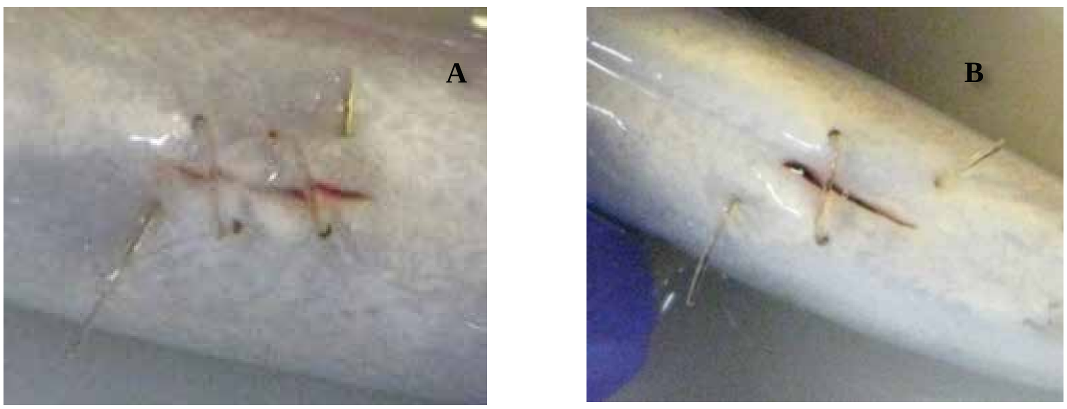

Figure 3. Day 0 Suture Patterns Demonstrating the Final Product of the 6-Point (A, left photo) and Wide "N" (B, right photo) Schematics and Descriptions. The Wide "N" Knot (for both 12 and 18 mm) has a similar pattern to the Wide "N" with large angles between and fewer entry/exit points (photo B) than the 6-Point pattern (photo A; see Section 2.2, Figure 2 for pattern mechanics).[1]

Fish receiving surgical implants (PIT and AT) were placed on the surgery table and given a maintenance anesthetic dose (river water containing 40 mg/L of MS-222) through silicone rubber tubing from a gravity-fed bucket. The surgeon controlled the dose during the procedure by mixing river water with the maintenance anesthetic water. With the fish ventral side up, a 4- to 5-mm incision was made along the linea alba, between the pectoral fin and pelvic girdle. Incisions were closed using an absorbable bi-directional knotless monofilament tissue-closure device (MonodermTM, QuillTM, Angiotech Pharmaceuticals, Vancouver, BC). The suture patterns and approach for insertion are described in Section 2.2, Suture Patterns. After surgery, a photo was taken of the closed incision and fish were placed in fresh aerated water to recover. Once fish regained equilibrium they were placed in one of two circular tanks and provided with flow-through river water. Over the 14 days of holding, water temperature ranged from 19.8 to 22.5 °C (average temperature = 21.5 ± 0.49°C).

[1] The suture ends are longer in the photos to be visible to the reader; the ends should be no longer than 3 mm.

2.4 Response Examinations

Mortalities and tag loss were monitored on a daily basis. The ATs and PITs were only scored as lost if the tag completely exited the fish. Incision responses for all fish were examined 7 and 14 days after surgery (herein referred to as day 7 and day 14). Each fish was anesthetized with 80 mg/L of MS-222 for examination. Fish were removed from the bath, fork length (mm) and wet weight (g) were measured, and the fish were placed on a foam pad, ventral side up. Maintenance anesthetic of up to 40 mg/L of MS-222 was supplied to the fish in the same manner as for surgery. The incision, suture, and surrounding area were examined through a stereomicroscope (0.65x magnification; Stemi 2000-CS; Zeiss AG, Jena, Germany) connected to a computer.

The incision area was partitioned into paired suture sites, i.e., having an entry and exit point pair (Figure 2). The 6-Point configuration had three sites while the Wide “N”, Wide “N” Knot 12, and Wide “N” Knot 18 had two sites each. Photographs of the fish were taken and the area of incision openness (mm^2), ulceration (mm^2), and redness (mm^2) were outlined and quantified using the “Image J” image processing program (public domain software, National Institute of Health, Bethesda, MD, http://rsb.info.nih.gov/ij/) (Figure 4). If there was more than one area on the fish with openness, ulceration, or redness, individual measurements were summed for the analyses. On days 7 and 14 the presence of suture material was noted for each site and marked as a binary response (present “1” or absent “0”), and for suture tension consistency (yes or no). Sutures were deemed non-functional if they were absent or lacked tension to properly close the incision. The suture functionality index was compared to incision openness.

On day 14, all fish were necropsied and biocompatibility effects were evaluated—effects such as the presence of tag bulge and fibrous tissue. Tag bulge for each treatment was marked as a binary response (present “1” or absent “0”). The presence of fibrous tissue was marked as a binary response (present “1” or absent “0”) and was scored based on severity (absent, minor, or encapsulation). At the end of the study, all observations were ranked for each treatment to give an overall performance index (1 = best and 4 = worst).

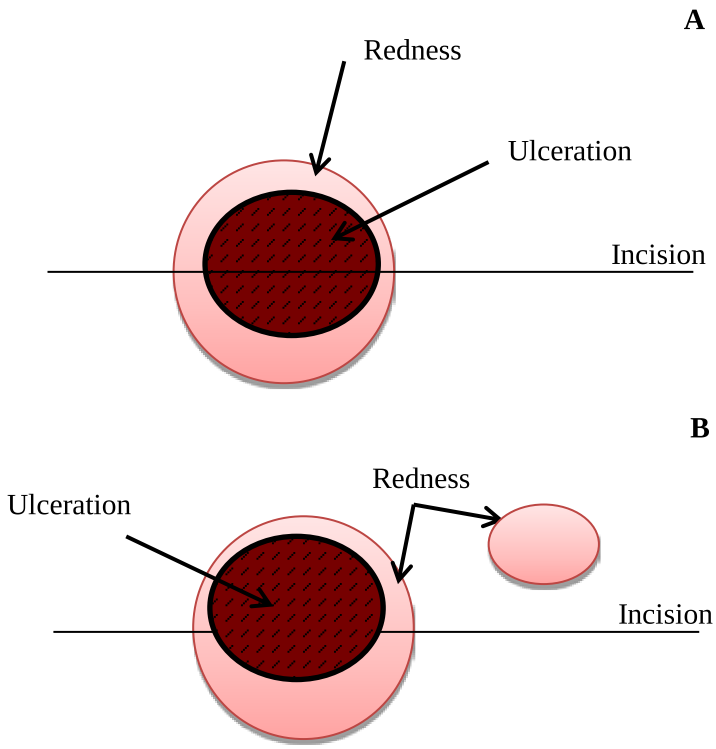

Figure 4. Wound Redness and Ulceration Differentiation. Redness was differentiated from ulceration by the consistency of the wound and area affected. In the above diagram, redness scores would include the pink area, not the maroon-hashed area. Ulceration scores would include the maroon-hashed area (inner circles in A and B) and not the pink areas. A) Only the pink area outer ring would be included in the redness score. Any redness in the ulcerated area was included in the ulceration score, not the redness score. B) The redness score would include the pink areas for each noted wound or affected area by adding the total pink areas together. Ulcerations, if more than one, would be summed similarly. This approach allowed for the distinction between red inflamed areas and areas with exposed underlying tissue.

2.5 Statistical Analysis

Categorical covariates included four suture treatments (6-Point, Wide "N", Wide "N" Knot 12, and Wide "N" Knot 18) and two exam days (day 7 and day 14). The response variables—mortality, tag retention, and functional suture (suture presence and tension)—at exam day and at necropsy were treated as binomial data, because the variable could either be present or absent in each fish. The variables redness, ulceration, and openness were continuous data. For questions 1, 2, 4, and 6 (Section 1.1), the response variable was categorical. For these questions, a Fisher's Exact Test (FET) was used to test for an association between the four suture treatments and the categorical response variable. For question 7 the response variable was continuous, so Analysis of Variance (ANOVA) was used to test for differences between treatments. For question 3 and 5, the response variable was measured as a categorical and continuous response, so both FET and ANOVA were used.

3.0 Results

Fish size and results related to the effects of suture pattern or type are described in the following sections. Mortality rate, AT or PIT retention, incision openness (gaping), suture functionality, occurrence of redness and/or ulceration, tag bulging, and fibrous tissue development are considered and ranked according to a performance index.

3.1 Fish Size

Subyearling Chinook salmon fork length (FL) ranged from 115 to 133 mm ($\bar{x}$ =122.67 ± 5.2 mm FL) and wet weight (WW) ranged from 10.1 to 22.7 g ($\bar{x}$ = 15.46 ± 3.18 g; Figure 5). FL was a significant predictor of WW (N = 21, $F(1, 19) = 75.2639$, $P < 0.0001$). The linear relationship between FL and WW can be described as WW = -50.50436 + 0.5377685*FL ($R^2 = 0.7984$; Figure 6). WW was confirmed as non-significant confounding variable in the experiment, so that the fish could be pooled for the following analyses (N = 21, $F(3, 17)= 0.2484$, $P = 0.8613$).

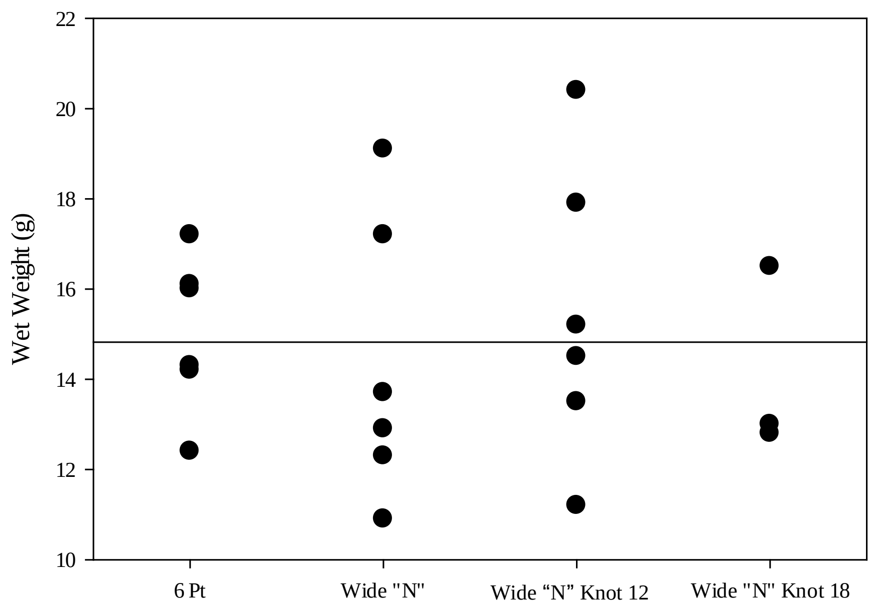

Figure 5. Wet Weight (g) of Study Fish for Each Treatment. Each circle (●) represents an individual fish; the black line (-) is the overall mean.

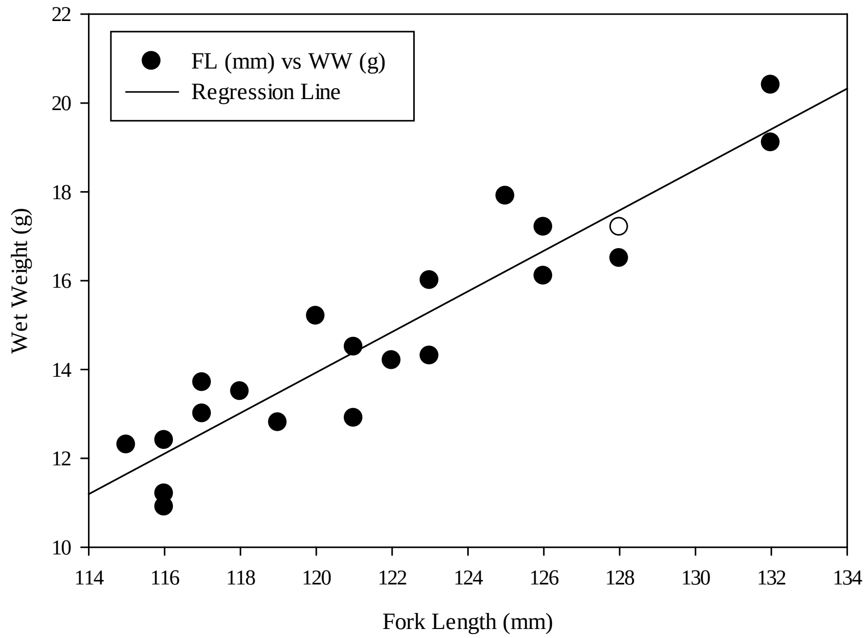

Figure 6. Fork Lengths (mm) and Wet Weights (g) of Study Fish. Each circle (●) represents an individual fish.

3.2 Mortalities

To address whether suture pattern and type influenced mortality rates, we examined the mortality frequency among treatments. Overall experimental mortality was low; only one fish was removed from the 6-Point treatment group on day 10 (Table 2). This fish had a large ulcerated wound on its right flank

that was not directly related to the incision, suture entry/exit points, or suture material. On day 7, this fish was reported as having a 2.73-mm2 incision gap, minor redness, and ulceration on site 2 (0.18 and 0.27 mm2, respectively), and no signs of ischemia. No other study fish exhibited similar wounds or other signs of illness. No difference in mortality was detected between treatment types (N = 1, P = 1.0, FET). There were no mortalities in the Control group; therefore, controls were excluded from further analyses.

Table 2. Mortality Frequency for Each Treatment

Mortality	6-Point	Wide “N”	Wide “N” Knot 12	Wide “N” Knot 18
No	5	6	6	3
Yes	1	0	0	0

3.3 Tag Loss

To address whether one suture pattern and type had a greater rate of tag loss, we analyzed the number of lost ATs and PITs. AT loss was relatively high (14.3%). Two fish in the Wide "N" treatment configuration dropped ATs between days 5 and 10 (Table 3). A fish in the Wide "N" Knot 12 treatment group had a hanging tag protruding through the incision on day 14. This fish was not included in the tag loss count because the AT had not released from the incision. A fish in the Wide "N" Knot 18 treatment group expelled its AT on day 11; this fish had the greatest incision openness (7.10 mm^2) for this treatment group on day 7, but the incision openness was 0 mm^2 on day 14. There were no differences in AT retention rates among treatments (N= 20, $P = 0.6211$; FET) by day 14. This was likely due to very low statistical power as a result of small sample sizes. Fish size was not a significant factor in the loss of ATs (FL: N = 20, $X^2 = 0.5037$, $P = 0.4139$; WW: N = 20, $X^2 = 0.0706$, $P = 0.7905$; Logistic). There were no dropped PITs; therefore statistical analyses were not performed.

Table 3. AT Retention for Each Treatment Group. Frequency of occurrence as a percentage is shown in parentheses for each treatment.

Tag Retention	6-Point	Wide "N"	Wide "N" Knot 12	Wide "N" Knot 18
Not Dropped	5	4	6	2
Dropped	0 (0%)	2 (33%)	0 (0%)	1 (33%)

3.4 Incision Openness

To determine whether one suture pattern or type had a greater influence on tag loss or incision healing, we examined incision openness (surface area; mm^2) on days 7 and 14. On day 7, Wide "N" had the greatest mean openness among the treatments. Fish in the Wide "N" treatment group that dropped ATs had incision openness areas of 1.53 and 6.33 mm^2 on day 7, and 0 mm^2 on day 14 (Table 4). There were no significant differences in the incision openness on day 7 among the treatment groups (N = 21, $F(3, 17) = 1.008$, $P = 0.4132$, Figure 7). On day 14, only 1 fish in the Wide "N" Knot 12 treatment group had an open incision (7.12 mm^2). The large openness is attributed to the AT that was protruding halfway through the incision. Similar to day 7, there were no significant differences among treatment groups on day 14 (N = 20, $F(3,16) = 0.7467$, $P = 0.5399$, Figure 7).

Table 4. Incision Openness (mm^2) on Days 7 and 14 by Suture Type. Average incision openness ± SD and frequency of occurrence as a percentage is shown in parentheses for each treatment.

Observation Day	6-Point	Wide "N"	Wide "N" Knot 12	Wide "N" Knot 18
7	0.59 ± 1.10 (33%)	2.86 ± 3.01 (67%)	1.05 ± 2.17 (33%)	2.37 ± 4.10 (33%)
14	0 (0%)	0 (0%)	7.12 ± 0.0 (17%)	0 (0%)

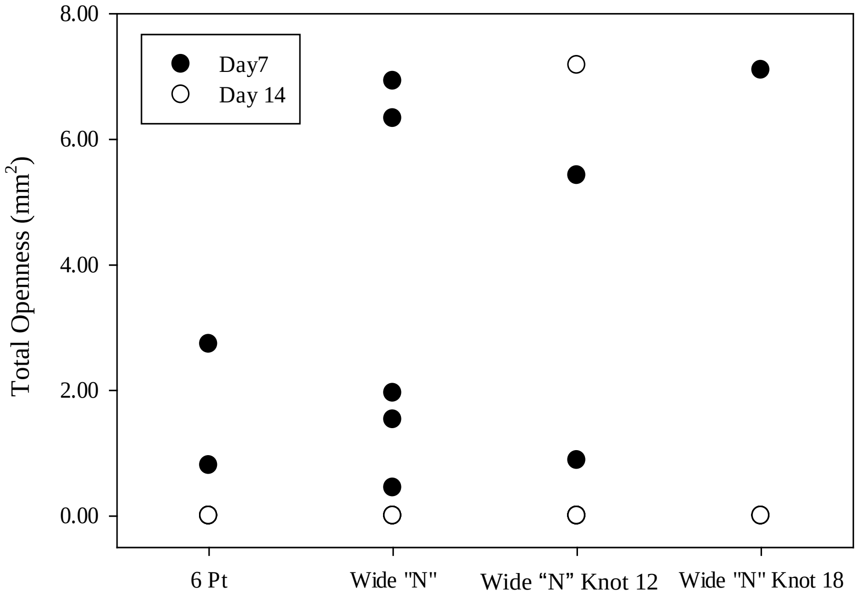

Figure 7. Total Incision Openness (mm^2) by Treatment. Day 7 is represented by the filled circles (●); day 14 is represented by the open circles (○). Data points overlap at 0.00 mm^2.

Fish size was not a significant predictor of incision openness on day 7 (FL: N = 21, $F(1, 19) = 0.0226$, $P = 0.8820$; WW: N = 21, $F(1, 19) = 0.2542$, $P = 0.6199$; Figure 8). No statistical analyses were conducted on day 14, because only one fish demonstrated incision openness, due to AT protrusion.

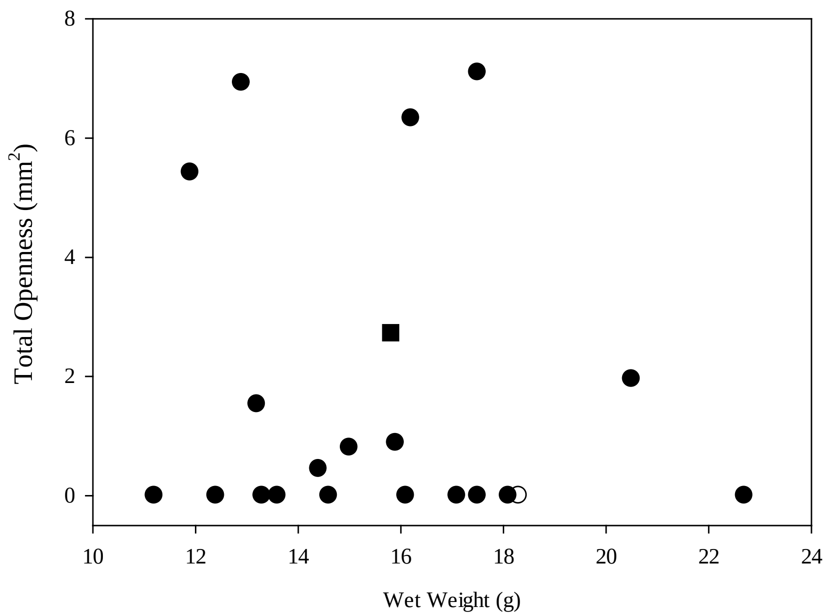

Figure 8. Total Incision Openness (mm^2) as a Function of Wet Weight, Day 7. WW was likely not a determinative factor in the openness. The square symbol (■) represents the day 7 score of the one mortality that occurred on day 11. Data points overlap at 0.00 mm^2.

3.5 Functional Suture

Because the bi-directional closing devices do not have knots that close the incision in traditional loops, we evaluated the presence and tension of each suture exit and entry point across the wound as functional or non-functional (see Figure 2 for site numbers). During necropsy, some of these sutures were found within the fish cavity or imbedded in the tissue.

At site 1 on day 7, the fish in the 6-Point and Wide "N" treatment groups had no functional sutures, while 50% and 67% of the fish in the Wide "N" Knot 12 and Wide "N" Knot 18 treatment groups, respectively, had functional sutures (N = 21, P = 0.0400; FET; Table 5). On day 14, fish in the 6-Point and Wide "N" treatment groups had no functional sutures, while fish in the Wide "N" Knot 12 and Wide "N" Knot 18 treatment groups had significantly more functional sutures, 33% and 67%, respectively (N = 20, P = 0.0358; FET).

At site 2 on day 7, there were was no significant difference in the number of functional sutures among treatments (N = 21, P = 0.1028; FET; Table 5), because fish in the 6-Point, Wide "N" Knot 12, and Wide "N" Knot 18 treatment groups had functional sutures remaining (17%, 33%, and 67% respectively; Table 5). At site 2 on day 14, fish in the 6-Point and Wide "N" treatment groups had no functional sutures, while those in the Wide "N" Knot 12 and Wide "N" Knot 18 treatment groups had significantly more functional sutures, 33% and 67%, respectively (N= 20, P = 0.0358; FET; Table 5).

No statistical analyses were conducted on the third entry/exit point (site 3) because the 6-Point treatment was the only treatment with three sites (Table 5).

Table 5. Number of Functional Sutures by Entry/Exit Site for Each Treatment Group. The last two rows indicate the presence of the suture internally, either in the body cavity or embedded in tissue.

Observation Day	Site	Functional at Entry/Exit Site	6-Point	Wide "N"	Wide "N" Knot 12	Wide "N" Knot 18
7	1	Yes	0	0	3	2
	1	No	6	6	3	1
	2	Yes	1	0	2	2
	2	No	5	6	4	1
	3	Yes	2	NA	NA	NA
	3	No	3	NA	NA	NA
14	1	Yes	0	0	2	2
	1	No	5	6	4	1
	2	Yes	0	0	2	2
	2	No	5	6	4	1
	3	Yes	0	NA	NA	NA
	3	No	5	NA	NA	NA
Necropsy		Suture present	0	1	2	2
		Suture not present	5	5	4	1

NA = not applicable

On day 7, the functional suture index was not significantly related to total incision openness (N = 21, $F(2, 18) = 1.0914$; $P = 0.3570$). Conversely, on day 14, the functional suture index was significantly related to incision openness (N= 20, $F(2, 17) = 3.6125$, $P = 0.0493$). These contradictory results are likely influenced by the low statistical power.

3.6 Ulceration and Redness

To determine whether suture pattern or type influenced tag loss or physiological stress, we examined the number of redness and ulceration occurrences and their total surface area by treatment group (Figure 9). Simplifying the analysis to presence or absence of ulceration, on day 7 the 6-Point treatment group had significantly more ulceration (N = 21, $P = 0.0092$, FET; Table 6), followed by Wide "N" Knot 12 (50%fish with ulceration), Wide "N" Knot 18 (33% fish with ulceration), and Wide "N" (17% fish with ulceration) groups. However, total ulceration surface area around the incision and/or the suture entry/exit sites (surface area measurements, mm^2) was not significantly different among treatments (N = 21, $F(3, 17) = 1.6395$, $P = 0.2199$; ANOVA; Table 6).

Using a similar approach on day 14, the simplified analysis of ulceration presence or absence for each fish indicated that the Wide "N" Knot 18 treatment group had significantly more ulceration (67% fish with ulceration) than the Wide "N" Knot 12 (33% fish with ulceration), Wide "N", and 6-Point (N = 20, $P = 0.0158$, FET; Table 6) groups. The total ulcerated surface area around the incision and/or the suture entry/exit sites (surface area measurements, mm^2) by day 14 had lessened in three of the treatments (Table 6), but the Wide "N" Knot 18 treatment group showed a slight increase. There was no significant difference among treatments (N = 20, $F(3, 16) = 2.1859$, $P = 0.1295$; ANOVA; Table 6).

Table 6. Frequency and Mean Area of Ulceration for Each Treatment. The total mean and standard deviation of ulcerated area (mm^2) are provided in the row for each day.

Observation Day	Ulceration	6-Point	Wide "N"	Wide "N" Knot 12	Wide "N" Knot 18
7	Yes	6	1	3	1
	No	0	5	3	2
	$\bar{x}$ mm^2 ± SD	1.99 ± 2.28	0.02 ± 0.06	1.04 ± 1.73	0.25 ± 0.36
14	Yes	0	0	3	2
	No	5	6	3	1
	$\bar{x}$ mm^2 ± SD	0.00 ± 0.00	0.00 ± 0.00	0.29 ± 0.41	0.30 ± 0.29

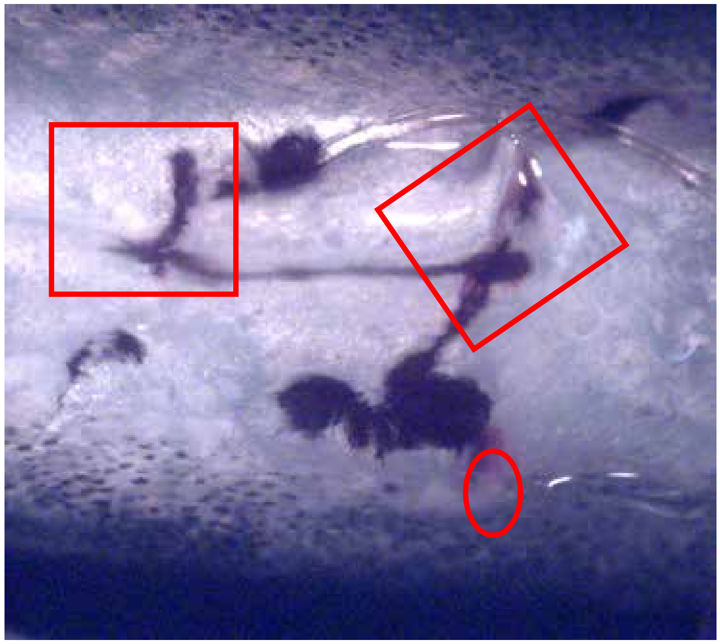

Figure 9. Example of Ulceration and Redness Due to Suture Tearing. The red circle (○) highlights redness not directly incorporated with ulceration. The red squares (□) denote ulceration and redness that were separated using Image J. The circle and square do not denote the actual Image J patterns and measurements used for the final summations of total ulceration and redness.

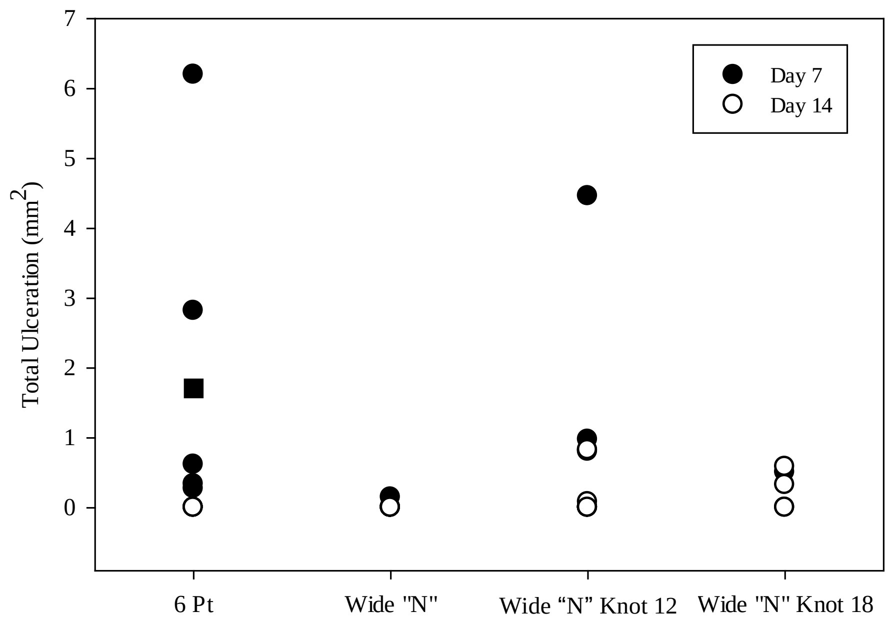

Figure 10. Total Ulceration (mm^2) as a Function of Treatment. Day 7 is represented by the filled circles (●); day 14 is represented by the open circles (○). The square symbol (■) represents the day 7 score of the fish that died. Data points overlap at 0.00 mm^2.

When simplifying the analysis to the presence or absence of redness for each fish, significant results were found (*P* = 0.0199, FET; Table 7) on day 7. Fish in the 6-Point and Wide "N" Knot 18 treatment groups had significantly more redness (N = 21, $P = 0.0199$, FET, Table 7) than those in the Wide "N" Knot 12 (67%) and Wide "N" Knot 18 (33%) treatment groups. Similarly, total redness surface area (mm^2) around the incision and/or the suture entry/exit sites was significantly greater in fish in the Wide "N" 18 treatment group than in the Wide "N" treatment group (N = 21, $F(3, 17) = 3.2031$, $P = 0.0497$; ANOVA, Honestly Significant Difference; Table 7).

Using the simple analysis of the presence or absence of redness for each fish, no significant results were found among treatments (N = 20, $P = 0.6153$, FET; Table 7, Figure 11) on day 14. A total of 67% of the Wide "N" Knot 18 treatment fish had redness, followed by the Wide "N" and Wide "N" Knot 12 treatment fish, 37% of which exhibited redness, and the 6-Point treatment fish, 20% of which showed redness. Total redness surface area (mm^2) around the incision and/or the suture entry/exit sites by day 14 had lessened in all but the Wide "N" Knot 12 treatment group (Table 7). There were no significant differences in the measured total redness area among treatments (N = 20, $F(3, 16) = 0.7746$, $P = 0.5251$; ANOVA; Table 7, Figure 11).

Table 7. Frequency and Mean Area of Redness for Each Treatment

Observation Day	Redness	6-Point	Wide "N"	Wide "N" Knot 12	Wide "N" Knot 18
	Yes	6	2	4	3
7	No	0	4	2	0
	$\bar{x}$ mm^2 ± SD	1.24 ± 1.66[(a)]	0.14 ± 0.25	0.25 ± 0.23[(b)]	5.88 ± 7.83[(a)]
	Yes	1	2	2	2
14	No	4	4	4	1
	$\bar{x}$ mm^2 ± SD	0.00 ± 0.00	0.02 ± 0.04	0.39 ± 0.88	0.12 ± 0.15

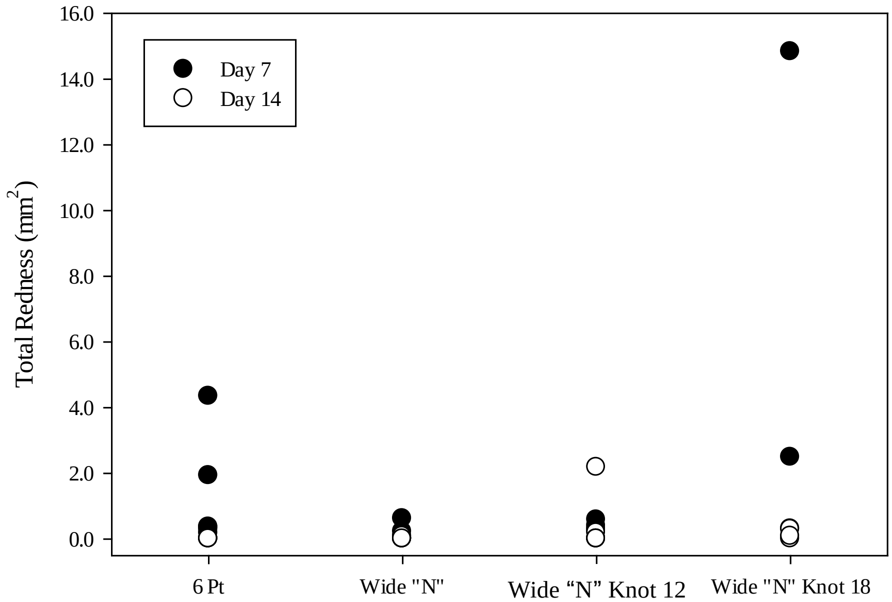

Figure 11. Total Redness (mm^2) by Treatment. Day 7 is represented by the filled circles (●); day 14 is represented by the open circles (○). The square symbol (■) represents the day 7 score of the fish that died (0.17 mm^2). Data points overlapped at 0.00 mm^2.

3.7 Transmitter Bulge and Fibrous Tissue

The effects of suture patterns and fish size were examined for their influence on AT bulging and fibrotic tissue development. AT bulging, visible during the necropsy stage, was not significantly related to fish size (N = 20, X^2 = 1.078, P = 0.2991; Logistic) or treatment (N = 20, P = 0.4588; FET; Table 8).

Table 8. Frequency of AT Bulge Observed During Necropsy Examinations

AT Bulge	6-Point	Wide "N"	Wide "N" Knot 12	Wide "N" Knot 18
Absent	4	6	5	3
Present	1	0	1	0

The occurrence of fibrous tissue was not significantly different among treatments (N = 20, P = 0.6153; FET; Table 9), nor was the severity of fibrotic tissue significantly different among treatments (N = 20, P = 0.7396; FET). Fish WW was not a significant variable in the severity of fibrotic tissue (N = 20, X^2 = 0.0384, P = 0.8447; Figure 12).

Table 9. Frequency of Fibrotic Tissue Observed During Necropsy Examinations

Fibrotic Tissue	6-Point	Wide "N"	Wide "N" Knot 12	Wide "N" Knot 18
Absent	1	2	2	2
Minor	2	1	2	0
Encapsulation	2	3	2	1

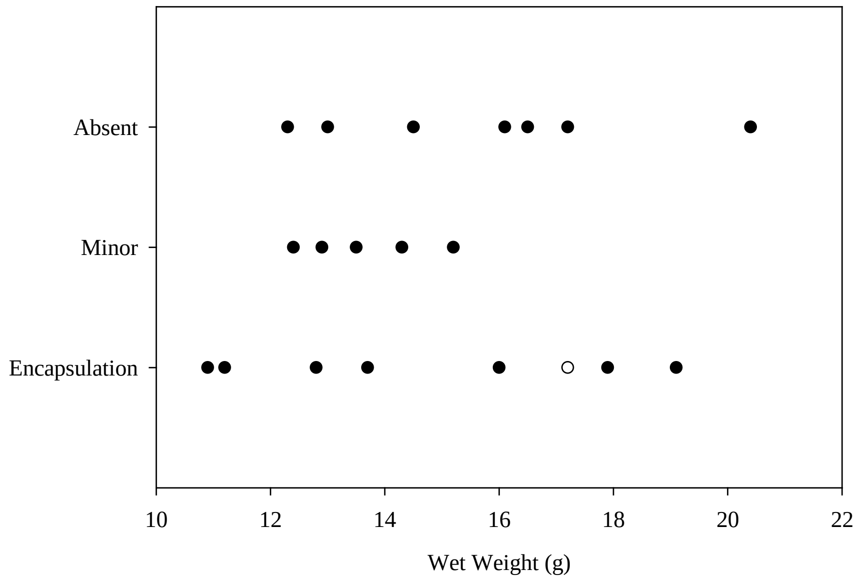

Figure 12. Fibrous Tissue Development as a Function of Wet Weight (g)

3.8 Performance Index

Each characteristic of interest was ranked to assist with recommendations based on the performance of each pattern (Table 10). None of the suture patterns or types performed significantly differently from the others (N = 44, $P = 0.2311$; FET).

Table 10. Performance Index Based on Rank of Each Measured Treatment Observation

	Treatment			
Measured Observation	6-Point	Wide "N"	Wide "N" Knot 12	Wide "N" Knot 18
Mortality	4	2	2	2
ATs Dropped	2	3.5	1	3.5
Presence of Gaping, day 7	3	3	1	3
Functional Suture, Site 1, day 7	3.5	3.5	1	2
Functional Suture, Site 2, day 7	3	3	1	3
Presence of Ulcerated Areas, day 7	4	1	3	2
Presence of Ulcerated Areas, day 14	1.5	1.5	3	4
Presence of Redness Areas, day 7	1.5	3	4	1.5
Presence of Redness Areas, day 14	1	2.5	2.5	4
AT Bulge	3.5	1.5	3.5	1.5
Fibrous Tissue	3	3	3	1
Average	2.73	2.50	2.27[a]	2.50

1 = best, 4 = worst
(a) Treatment with the lowest overall score and thus the overall best performance.

4.0 Discussion

The objective of this study was to assess the performance of a bi-direction knotless tissue-closing device on ROR SYC implanted with ATs and PITs, using absorbable monofilament material in three suture patterns and needles of two different circumferences. We examined eight categorical factors including survivorship, AT loss, incision openness, functional suture (including presence and tension across wound), ulceration, redness, tag bulging, and tissue fibrosis. Finally, we incorporated the frequency of occurrence for each factor by treatment group to determine an average performance ranking (1 to 4). The results may be confounded by small sample size and thus low statistical power on most tests. Although some categories had significant differences among treatments, the performance index indicated that the Wide "N" Knot 12 treatment group overall performed *better* than the other treatment groups, although it was not consistently superior.

One mortality occurred during the experiment—the 6-Point treatment group had a 17% mortality rate, which was low compared to concurrent experiments using actively outmigrating SYC (Brown et al. 2010; Woodley et al. 2011). The mortality rate in this study was 11% lower than that observed after 14 days of a tag expulsion study for SYC surgically implanted with ATs and PITs (Woodley et al. 2011). The SYC in this study were from the same sampling set (07/19/2010) and were exposed to similar tank and ambient water conditions. Therefore, the mortality rate of 17% observed in the 6-Point treatment group may not be an effect of treatment, rather an example of fish in poor condition given the time of year, water temperatures, and individual progression into their smoltification cycle (Woodley et al. 2011).

There were no dropped PITs in any of the treatment groups, although the Wide "N" and Wide "N" Knot 18 treatment groups had an AT loss rate of 33%. A single fish in the Wide "N" Knot 12 treatment group had an AT bulging through the incision by day 14, but technically it had not dropped. The 6-Point treatment should have the advantage of greater and more uniform tension across the incision due to the increased number of entry and exit sites, which may have prevented the loss of ATs and PITs because the suture tended to stay functional in the middle site (#2, see Figure 2). Deters et al. (2010) obtained 94% and 82% suture retention on day 7 and day 14, respectively, using two interrupted sutures when comparing seven suture materials. With the exception of the 6-Point treatment, the bi-directional knotless suture using the tested patterns allowed for a greater rate of AT loss than the current suture material and pattern approach.

When Monocryl™, the current recommended monofilament, does not have proper tension across the wound, the suture has a higher chance of poor apposition, and subsequently slower healing (Lin et al. 1996; Wagner et al. 2000; Bridger & Booth 2003; Fortenot & Neiffer 2004). Given that a bi-directional knotless suture should have more uniform tension across the incision, we hypothesized that the chance of poor apposition would be reduced, because there would be less chance that the barbs would fail to engage in the tissue. For one measure of apposition—overlapping of incision flaps—the bi-directional knotless suture exceeded our expectations with no occurrence of overlapping. However, openness or incision gaping did occur. For example, on day 7, regardless of treatment group, the incisions on a few fish exhibited some amount of openness; the 6-Point treatment fish had the least openness, which was not related to fish size. By day 14, healing had occurred such that only one fish in the Wide "N" Knot 12 treatment group exhibited gaping, which was associated with the AT protruding through the incision. The increased tension across the wound and increased number of entry and exit sites was likely the reason fish in the 6-Point treatment group had less openness than those in the other treatment groups, even if sutures were not present in many of the fish by day 14. Overall, the openness of the incision in most cases was the result of the absence of the bi-directional knotless suture.

Most of the sutures, regardless of treatment type, had worked themselves out of the fish's tissue or were beginning to absorb in the fish by day 14. In the 6-Point treatment group, either site 1 or site 3 was not categorized as functional by day 7, and only two other sites exhibited functioning tension and apposition. By day 14, there were no functional sutures in the 6-Point treatment fish and no sutures were observed during necropsy. Similarly, by day 7 the Wide "N" treatment fish had no functional sutures, although one fish retained the sutures. Because of the anchoring of the knot in the Wide "N" Knot 12 and Wide "N" Knot 18 treatment groups, 33 to 66% of sites 1 and 2 were still functional on days 7 and 14. The absence of sutures occurred at a higher frequency than expected based on prior research (Deters et al. 2010; Panther et al 2010). The sutures used tended to be more rigid than traditional monofilament, which may have contributed to the sutures working themselves loose when not kept at warmer temperatures (i.e., 30°C and above), and thus forced themselves out of the desired suture pattern and out of the fish.

Ulceration and redness occurred in all treatment groups on both examination days. In most cases, the ulceration was directly related to the sutures tearing through the tissue towards the incision. The Wide "N" treatment group had the least amount of ulceration by day 7, followed by the Wide "N" Knot 18, Wide "N" Knot 12, and 6-Point treatment groups. The number of fish with ulceration and/or redness coincided with the number of fish that had sutures visibly present on day 7. This result was contrary to the purpose of the barbed suture, which was to distribute tension across the incision more evenly and minimize tissue tearing. The "tearing" of tissue observed was related to 1) the drag created by the suture hanging out of the fish (Figure 13A); 2) tissue bunching resulting from the barbs moving during the swimming action of the fish (Figure 9, Figure 13b); and 3) the barbs tearing the tissue immediately around the entry/exit points, eventually causing the suture to fall out of the fish (Figure 13C).

Suture presence, ulceration, and redness occurrence may be confounding factors. For example, ulceration occurred in all 6-Point fish on day 7, when the sutures were still present and most were not functional. By day 14, the ulceration had healed; however, no sutures were present. Similarly, on day 7 the Wide "N" treatment group had only one fish with ulceration and no functional sutures, although a few sutures remained present in the fish. By day 14, the ulceration had healed, and there were no functional sutures present. Both the Wide "N" and Wide "N" Knot 12 fish had ulcerations on both days 7 and 14, as well as functional sutures on both examination days. Measure of redness followed similar patterns, in that by day 14 the 6-Point and Wide "N" fish had nearly no (0 mm^2) redness, while fish with functional sutures had more redness and ulceration.

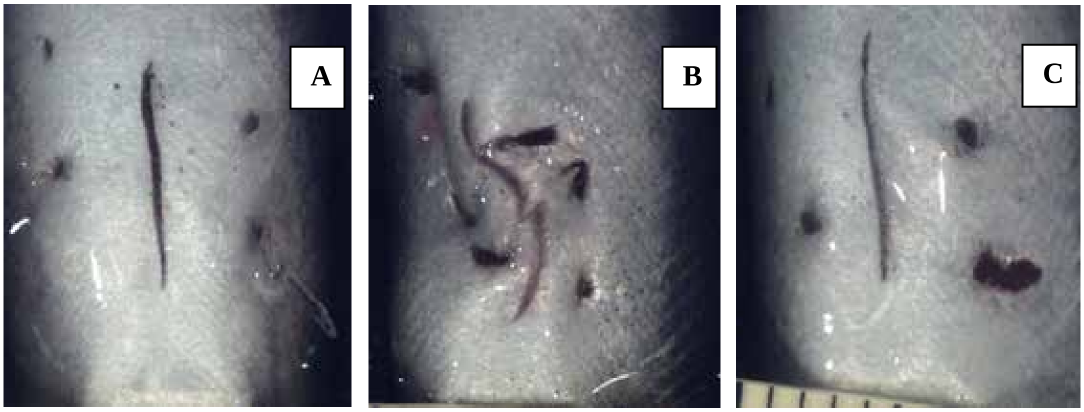

Figure 13. Photos Taken from Day 7 Response Examinations that Show Ulceration and Redness Were Often Associated with the Barbed Suture Using the Wide “N” and 6-Point Suture Patterns. A) Wide “N” pattern in SYC where the suture has slipped out of the fish creating drag. B) 6-Point suture pattern where the suture is tightening, tearing the tissue towards the incision. C) Wide “N” pattern where the suture has slipped out or pulled into the fish leaving a torn or rubbed area associated with entry and exit points.

There was no indication that suture pattern or type increased the frequency of fibrotic tissue or AT bulge events. The 6-Point treatment group had a 22% occurrence of fibrotic tissue, while the other treatment groups had 33% occurrences. Conversely, the 6-Point treatment group had a 22% occurrence of tag bulge, while Wide “N” and Wide “N” Knot 18 groups had no occurrence and the Wide “N” knot 12 group had a 33% occurrence. There does not seem to be a good understanding why PITs and/or ATs tend to have tissue encapsulation associated with them.

The question remains whether bi-directional knotless tissue-closure devices are as effective as or more effective than traditional sutures for incision closure in juvenile Chinook salmon. At this time, we would not recommend using the tested patterns with 12- or 18-mm Monoderm™ bi-directional knotless sutures on juvenile SYC. Based on the suture retention and suture rigidity, bi-directional knotless sutures would likely be more suitable for use with large adult fish and/or fish with large scales. Several surgery factors should be considered prior to use in field conditions. Tissue type and suture geometry can influence retention/loss of the bi-directional knotless tissue-closure device (Ingle and King 2010). When the sutures are embedded in tissue there are two primary modes of failure—peeling or bending of the barb. Peeling occurs when the barb pulls away from the suture; bending occurs when the barb pulls back without breaking off. Bent barbs remain intact attached to the suture, but will eventually release from the surrounding tissue (Ingle and King 2010). A more flexible suture, barb geometry, or even number of barbs per suture may be required for better anchoring in juvenile Chinook salmon tissue.

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