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Performance Assessment of Bi-Directional Knotless Tissue-Closure Devices in Juvenile Chinook Salmon Surgically Implanted with Acoustic Transmitters, 2009

Final Report

CM Woodley
KA Wagner
AJ Bryson

November 2012

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Pacific Northwest National Laboratory
Marine Sciences Laboratory
Sequim, Washington 98382

Summary

The purpose of this report is to assess the performance of bi-directional knotless tissue-closure devices[1] for use in tagging juvenile salmon. This study is part of an ongoing effort at Pacific Northwest National Laboratory (PNNL) to reduce unwanted effects of tags and tagging procedures on the survival and behavior of juvenile salmonids, by assessing and refining suturing techniques, suture materials, and tag burdens.

The objective of this study was to compare the performance of the knotless (barbed) suture, using three different suture patterns (treatments: 6-point, Wide "N", Wide "N" Knot), to the current method of suturing (Monocryl™ monofilament, discontinuous sutures with a 2×2×2×2 knot) used in monitoring and research programs with a novel antiseptic barrier on the wound ("Second Skin"). This experiment was conducted at 12 and 17°C, which is similar to temperatures experienced in river.

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 the number of fish deaths per treatment group?
2. Does one suture pattern and its associated needle type yield better (i.e. greater) acoustic transmitter (AT) or passive integrated transponder (PIT) retention as measured by the number of dropped ATs or PITs?
3. Does one suture pattern and its associated needle type have a greater potential for tag loss and physiological stress as measured by incision gaping?
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 at Site 1, Site 2, and where it applies at Site 3?
5. Does one suture pattern and its associated needle type have a greater amount of tissue trauma as measured by wound ulceration and redness?
6. Is there transmitter bulging vary with fish size or suture treatment?
7. Is fish size a confounding variable to any of the above measures?

On October 14 and 15, 2009, juvenile subyearling Chinook salmon were implanted with Juvenile Salmon Acoustic Telemetry System (JSATS) micro-ATs (each 12 mm long × 5 mm wide × 4 mm high, 0.43 g in air) and PITs. Incisions were closed with either a bi-directional knotless tissue-closure device or Monocryl™ monofilament. The study was conducted at the PNNL Aquatics Research Laboratory. Test fish were examined on post-surgical days 7, 14, 21, and 28 for survival, tag loss, functional suture, incision openness, and redness and ulceration in the area of the incision. After external examination on day 28, fish were euthanized and necropsied for internal assessment of suture and tag effects.

The elevated ambient water temperature, 17°C, greatly affected all factors analyzed, thus likely indicating multiplicative stress from the surgeries and the long-term holding. The categorical factors examined significantly varied among suture pattern and treatment types and were expressed differently

[1] Monoderm™, Quill™, Angiotech Pharmaceuticals, Vancouver, BC.

between the two temperature treatments. Overall, in 12°C the performance index indicated that the Second Skin overall performed *better* than the other treatments, although not consistently superior across the examined factors. For the 17°C group, performance index indicated that the Wide "N" overall performed *better* than the other treatments, although not consistently superior across the examined factors. Both the Second Skin and Wide "N" treatments had more tags dropped than with the 6-Point treatment. For purposes of biotelemetry, in 17°C, the Second Skin treatment had the greatest gaping (days 21 and 28) and tag loss (overall), which may be of a concern when tracking juvenile Chinook in warmer water. We attribute this to the ability of the knotless (barbed) suture to maintain closure in the middle of the incision even when overall the ends of the suture were not functional. The simple interrupted suture pattern overlaid with the antiseptic does not provide structure or reliance to the middle of the incision, but rather to 1/3 and 2/3 across the incision. Conversely, the knotless (barbed) suture provided more structure initially across the whole incision as expected. However, as the suture became less functional, specifically the ends began to slide out of place; the middle section of the incision still remained closed with the device in place.

Ultimately, 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. Based on the suture retention and suture rigidity, bi-directional knotless sutures would likely be more suitable for use with larger adult fish and/or fish with large scales. Several surgery factors should be considered prior to use in field conditions. Tissue type or tissue consistency when exposed to thermal stress and suture geometry can influence retention/loss of the bi-directional knotless tissue-closure device (Ingle and King 2010; Jefferies et al. 2012). 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.

Acknowledgments

This project was funded by the U.S. Army Corps of Engineers (USACE), Portland, Oregon. We thank M. Brad Eppard, the USACE contracting officer's representative, for his oversight and guidance; and Richard S. Brown as the Project Investigator for the 2009 Tag Effects Study. We also thank the following Pacific Northwest National Laboratory staff and interns for their assistance with this research: Carmina Arimescu, Jim Boyd, Scott Carpenter, Thomas Carlson, Jessica Carter, Kathleen Carter, Kate Deters, Marybeth Gay, Kasey Knox, Tyrell Monter, Eric Oldenburg, Jennifer Panther, Andy Solcz, and Mark Weiland. Lastly, we would like to thank our thorough reviewer Amoret Bunn, editor Susan Ennor, and document production specialist Kathy Neiderhiser for their excellent comments and detailed editing.

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 (#2009-07), Battelle–Pacific Northwest Division. Trade names referenced do not imply endorsement by the U.S. Government.

Acronyms and Abbreviations

°C	degree(s) Celsius (or Centigrade)
AT	acoustic transmitter
ANOVA	analysis of variance
F	F-test statistic
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)
g	gram(s)
m^3	cubic meter(s)
mg	milligram(s)
mg/L	milligram(s) per liter
mm	millimeter(s)
mm^2	square millimeter(s)
MS-222	tricaine methanesulfonate
N	replicates
$NaHCO_3$	sodium bicarbonate
P	p-value; probability of test statistic
PIT	passive integrated transponder
rkm	river kilometer(s)
SD	standard deviation
SYC	subyearling Chinook salmon
WW	wet weight

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1.0 Introduction

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 an upregulated 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 an altered physical and physiological state of the tagged fish. Researchers at the Pacific Northwest National Laboratory (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 (Deters et al. 2010; Panther et al. 2010; Carter et al. 2011; Cooke et al. 2011).

In 2009, we began investigating the knotless (barbed) suture and suture patterns to determine if this technique is a viable alternative to the current method (Monocryl™ monofilament, discontinuous sutures with a 2×2×2×2 knot) used in river monitoring and research programs. On October 14 and 15, 2009, juvenile subyearling Chinook salmon (*Oncorhynchus tshawytscha*; SYC) were implanted with Juvenile Salmon Acoustic Telemetry System (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). A bi-directional knotless tissue-closure device (Monoderm™, Quill™, Angiotech Pharmaceuticals, Vancouver, BC) was used to close the incision. In this study, the effects of three suture patterns using the barbed suture material were examined over 28 days and compared to the currently accepted method of wound closure with a "Second Skin" (Cavilon™, No-Sting Barrier™, 3M, St. Paul, MN) applied over the wound. The study was conducted at PNNL's Aquatics Research Laboratory in Richland, Washington. Test fish were examined for suture and tag effects on post-surgical days 7, 14, 21, and 28. Fish were also examined for internal assessment of suture and tag effects on Day 28.

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 Federal Columbia River Power System (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 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 (ATs), 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 (Panther et al. 2010), or application of external mechanical forces, such as pressure (Stephenson et al. 2010). If transmitters are expelled, a false mortality rate occurs; 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 (Fontenot and Neiffer 2004; Harms 2005), or inefficient post-implantation recovery time (Harms 2005) can result in altered behavior, growth, and/or survival.

After insertion of 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 caused by 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; Jepson 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 synthetic monofilament compared to braided sutures in yearling juvenile Chinook (held at water temperatures of 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 mortality (Fontenot 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 Booth 2003; Fontenot 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

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[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. However, PNNL surgeries are conducted in a manner to be as "aseptic as possible."

to reduce tissue damage are needed to ensure the retention of intracoelomic transmitters, and reduce 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 entry and exits points of the suture coming through the tissue (Sadick et al. 1994; Shermak et al. 2009). Similar to synthetic absorbable monofilament, Monoderm™ is an absorbable monofilament (i.e., the copolymer material 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 ~45° from the main suture shaft (Figure 1.1). 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.

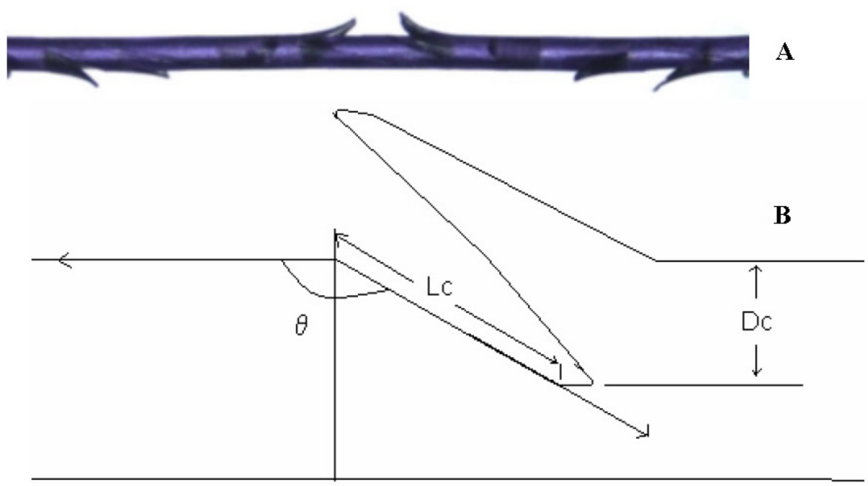

Figure 1.1. Knotless Suture Geometry. A) Knotless suture region where barbs transition from one direction to the other (accessed Angiotech March 15, 2011; http://www.angioedupro.com/Quill/index.php?ID=Photos). B) Individual barbs compared to the main suture shaft (photo credit and description Leung 2003).

1.2 Purpose and Scope

The objective of the study reported herein was to assess the performance of the bi-directional knotless tissue-closure device in relation to a currently accepted technique for wound closure in juvenile salmon. SYC were implanted with a JSATS AT and PIT, and the incisions were closed with separate treatments consisting of four suture patterns; three patterns using the knotless suture material and one suture pattern using Monocryl™ monofilament and covered with “Second Skin” (further described below). This study was conducted at water temperatures of 12 and 17°C, which are similar to those experienced in river. The wounds and suture performance were examined on 7, 14, 21, and 28 days post-surgery for suture loss, incision openness, 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 28th day post-implantation, fish were euthanized and necropsied to confirm the presence of the AT, PIT, and sutures, and to quantify the internal effects of tagging.

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 the number of fish deaths per treatment group?
2. Does one suture pattern and its associated needle type yield higher AT or PIT retention as measured by the number of dropped ATs or dropped PITs?
3. Does one suture pattern and its associated needle type have a greater potential for tag loss and physiological stress as measured by incision gaping?
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 at Site 1, Site 2, and where it applies at Site 3?
5. Does one suture pattern and its associated needle type have a greater amount of tissue trauma as measured by wound ulceration and redness?
6. Is there transmitter bulging vary with fish size or suture 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), results (Section 3.0), and discussion (Section 4.0). References for sources cited in the text are listed in Section 5.0.

2.0 Methods and Materials

This study, conducted over 29 days in fall 2009, involved fish acquisition, surgical implantation of ATs and PITs, examination of responses to implantation, and statistical analyses, as described below.

2.1 Fish Acquisition and Fish Maintenance

Subyearling Chinook salmon (N = 583) raised at the Priest Rapids hatchery and transferred to PNNL (Richland, WA) were used for this study. Fish were randomly sorted into one of two water temperature treatments; 12 or 17°C and held on a 12-h light to 12-h dark photoperiod during the acclimation (a 3°C change over 3 days) and experimental periods. Fish were housed in two 890-L circular fiberglass tanks supplied with aerated well water during acclimation for temperature treatments. Fish were fed Biodiet pellets (Bio-Oregon, Inc., Longview, WA) daily at a rate of 1.1% of their body weight. Food was restricted 24 h prior to and 6 h after surgery or weekly exams.

SYC 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. All methods were approved by the Institutional Animal Care and Use Committee (IACUC Protocol 2009-07).

2.2 Suture Pattern Mechanics

On the day of surgery, fish were assigned randomly to one of five treatments. All surgical treatments were performed using a 3/8 circle diamond point needle with 18-mm circumference. Treatment groups were as follows (Table 2.1):

- 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, and involved pulling the suture through opposing sides and ensuring the barbs were anchored in both directions.
- 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 and involved pulling the suture through the opposing sides and ensuring barbs were anchored in both directions.
- Wide “N” Knot Suture treatment (herein referred to as Wide “N” Knot). This 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 rather than no knots as with Wide “N”. 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 caused by knot and suture tension.
- Two simple interrupted sutures were secured using a 2×2×2×2 knot pattern with Monocryl™ monofilament and Second Skin applied over the wound (herein referred to as Second Skin). This is the currently accepted technique for wound closure. Second Skin is a product used to create a

fast-drying, non-sticky barrier film that forms a breathable, transparent coating on the skin. The film is hypoallergenic, non-cytotoxic, and will not sting even when applied to damaged or denuded human skin.

- 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 treatment groups.

Table 2.1. Suture Patterns and Replicates of SYC in Each Treatment. All needles were 3/8 circle diamond point with an 18-mm circumference.

Temperature	Treatment	Knots Used	Number of Entry and Exit Points	Sample Size
12°C	6-Point	0	3	65
	Wide “N”	0	2	67
	Wide “N” Knot	1	2	66
	Second Skin	2	2	66
	Control	NA	NA	22
17°C	6-Point	0	3	69
	Wide “N”	0	2	66
	Wide “N” Knot	1	2	71
	Second Skin	2	2	69
	Control	NA	NA	22

Depending on the suture pattern, there are several entry and exit points. The 6-Point suture pattern shown in Figure 2.1A has two points where the needle entered the skin (point E_1, E_2) and four points where it exited the skin (points X_1, X_2, X_3, X_4). The first exit came from the needle that was passed into the cavity via the incision, exiting at point X_1 until the middle point of the barbed suture was halfway through the exit point. Next, the surgeon used the internal portion of the suture to exit at point X_2, cutting the suture 3 mm from the exit point (i.e., leaving a 3-mm tail). The suture remaining outside of exit point X_1 extended across the wound and entered the tissue at entry point E_1. The needle passed into the body cavity at point E_1 and extended across the wound exiting at point X_3, before extending across the wound and entering at point E_2 and exiting at point X_4. The excess suture at point X_4 was cut leaving a 3-mm tail (Figure 2.1A).

The Wide “N” pattern (Figure 2.1B) 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 X_1 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 X_2, and the remaining suture was cut 3 mm from the entry point, leaving a 3-mm tail. The remaining suture outside of exit point X_1 extended across the wound and entered the tissue at entry point E_1. The needle was passed back into the body cavity at point E_1 and extended across the wound at point X_3. The excess suture at point X_3 was cut leaving a 3-mm tail (Figure 2.2B).

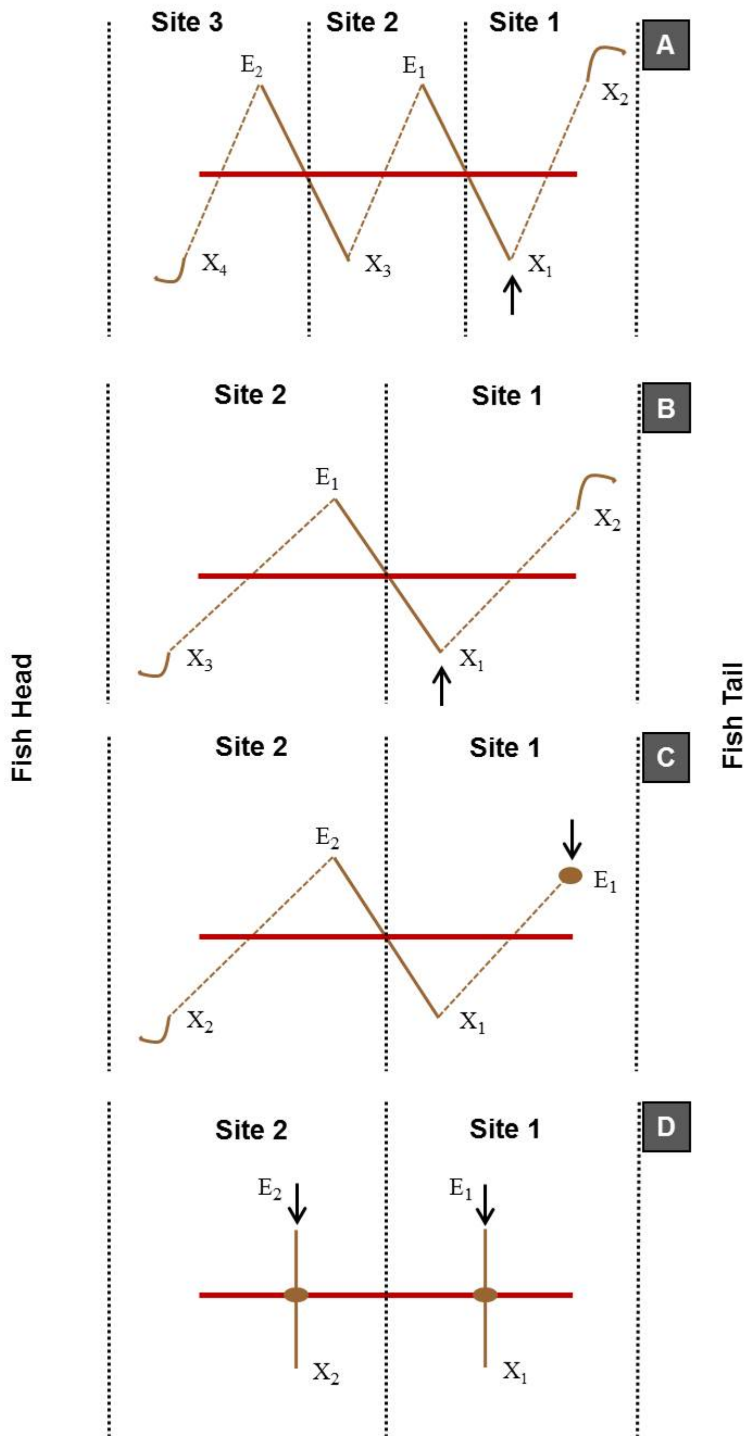

Figure 2.1. Schematic of the Four Suture Patterns. A) 6-Point, B) Wide "N", C) Wide "N" Knot, and D) Second Skin. Brown dashed lines represent internal suture areas. Brown solid lines represent external suture areas. Brown curved lines represent the needle. The brown solid dot is the knot tied for the Wide "N" Knot and Second Skin treatments. Black dotted lines section the incision into points for description purposes. The 6-Point pattern has three points, and the Wide "N", Wide "N" Knot, and Second Skin have two points. The arrows indicate the point of first insertion. Letters indicate entry (E_1-E_2) and exit (X_1-X_4) sites.

The Wide “N” Knot (Figure 2.1C) pattern used one segment of the suture with a knot tied at the end, denoted by the circle at point E_1. The needle passed through the body wall into the cavity at point E_1, exiting at point X_1, and the suture was pulled until the knot met the fish scales at point E_1. The needle was passed back into the body cavity at point E_2 and extended across the wound, exiting at point X_1. Excess suture was cut at point X_2.

The Second Skin pattern (Figure 2.1D) used two simple interrupted sutures with a knot for each tied in a 2×2×2×2 knot pattern. The 2×2×2×2 knot pattern consisted of four double throws in alternating directions. The needle made two separate entry (E_1 and E_2) and two separate exits (X_1 and X_2) and put pressure on two areas of the wound rather than evenly across the wound.

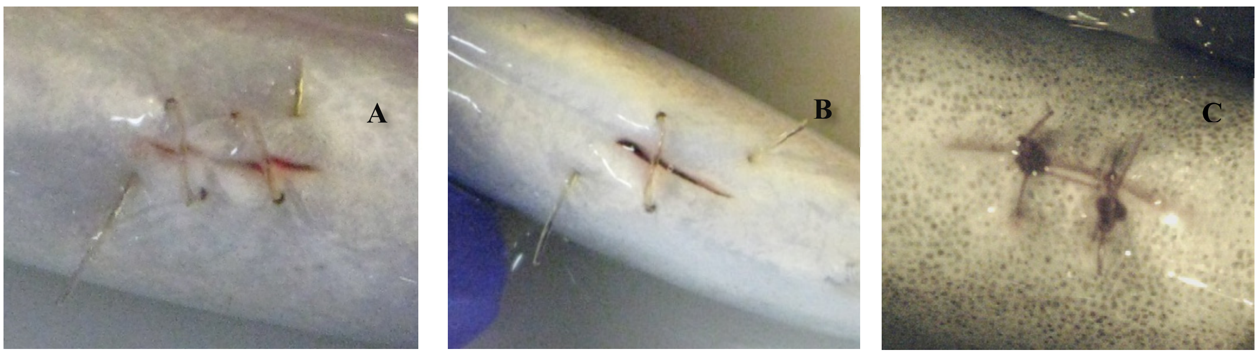

Figure 2.2. Day 0 Suture Patterns Demonstrating the Final Product of the 6-Point (A, left photo), Wide “N” (B, center photo), and the Second Skin (C, right photo). The Wide “N” and Wide “N” Knot suture patterns have a similar pattern with larger angles between sutures and fewer entry/exit points (photo B) than the 6-Point pattern (see Section 2.2, Figure 2.1 for pattern mechanics).[1]

2.3 Surgical Procedures

Surgeries on SYC were split into 2 days: October 14 and 15, 2009. Three surgeons performed all surgeries. Fish were anesthetized and handled similarly regardless of treatment. A buffered anesthetic (with 80 mg/L $NaHCO_3$) was prepared using aerated well 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 (WW; g), measured (FL, mm), and both flanks were photographed. Water temperature was monitored and new water was acquired if the temperature varied more than 2°C from the respective experimental temperature—12 or 17°C. Fish were randomly assigned to one of five treatment groups: 6-Point, Wide “N”, Wide “N” Knot, Second Skin, or Control. All suture treatment groups underwent surgical implantation, while the Control fish bypassed the surgery stations, and then were placed into 5-gal perforated recovery buckets (5 fish per bucket), aerated with well water, and monitored during recovery from anesthesia.

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

Fish receiving surgical implants (PITs and ATs) were placed on the surgery table and given a maintenance anesthetic dose (well water containing 40 mg/L of MS-222) through silicone rubber tubing from a gravity-fed bucket. Each surgeon controlled the dose during the procedure by mixing well water with the maintenance anesthetic water. With the fish ventral side up, a 5- to 7-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 (Monoderm™, Quill™, Angiotech Pharmaceuticals, Vancouver, BC) or Monocryl™ monofilament. 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 the fish regained equilibrium they were placed in one of two circular tanks and provided with flow-through well water. Over the experimental period, water temperatures fluctuated within one degree of the desired temperatures, 12 or 17°C.

2.4 Response Examinations

All fish were examined 7, 14, 21, and 28 days post-surgery (herein referred to as Day 7, Day 14, Day 21, and Day 28). Each fish was anesthetized with 80 mg/L of MS-222 for examination. Fish were removed from the bath, fork length (FL; mm) and wet weight (WW; 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 (0.65× magnification; Stemi 2000-CS; Zeiss AG, Jena, Germany) connected to a computer for photographing wounds.

The incision area was partitioned into paired suture points, i.e., having an entry and exit point pair (Figure 2.1). The 6-Point configuration had three points, while the Wide "N", Wide "N" Knot, and Second Skin had two points each. On days 7, 14, 21, and 28, the presence of suture material was noted for each point and marked as a binary response (present "1" or absent "0"), and for suture tension consistency (yes or no). The area of redness, ulceration, and incision openness (mm^2) were outlined and quantified using the "ImageJ" image processing program (public domain software, National Institute of Health, Bethesda, MD, http://rsb.info.nih.gov/ij/; Figure 2.3). If there was more than one area on the fish with either redness or ulceration, individual measurements were summed for the analyses. Sutures were deemed non-functional if they were absent or lacked tension to properly close the incision.

Redness was differentiated from ulceration by the consistency of the wound and area affected. Redness scores would include erythema (pink area in Figure 2.3), not ulcerations (maroon-hashed area, Figure 2.3). Ulceration scores would include the maroon-hashed area (inner circles in A and B, Figure 2.3) but exclude redness (pink area, Figure 2.3). Redness and/or ulcerations, if more than one occurrence per fish, would be summed (i.e., redness in Figure 2.3, two area would be summed into a single value). This approach allowed for the distinction between red inflamed areas and areas with exposed underlying tissue.

Throughout the study, fish were randomly sacrificed for future histological examination. These fish were removed from any further analyses. All other fish were necropsied after the Day 28 examination. On Day 28, suture presence was noted (present or absent), as well as any tag bulging. At the end of the study, all characteristics were ranked to give an overall performance index for each treatment (1 = best and 4 = worst). See Table 2.2 and Table 2.3 for a full list of sample sizes for incision openness, ulceration, and redness analyses with fish removed for mortalities or histological examination.

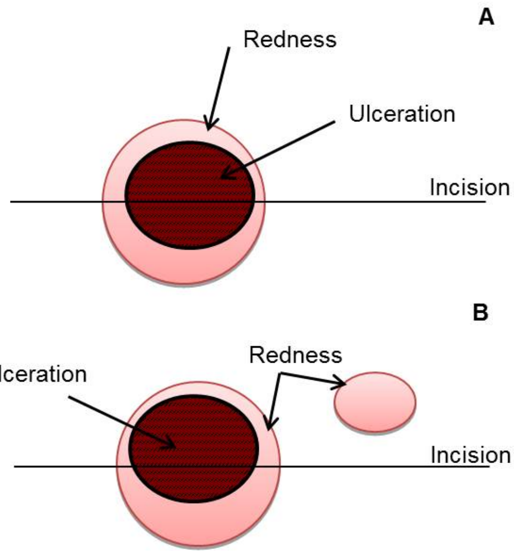

Figure 2.3. Wound Redness and Ulceration Differentiation. A) Only the pink area outer ring would be included in the redness score. Any redness in the ulcerated area was included in the ulceration score, not the redness score. B) The redness score would include the pink areas for each noted wound or affected area by adding the total pink areas together. Ulcerations, if more than one, would be summed similarly.

Table 2.2. Sample Sizes for Each Treatment at 12°C by Sample Day. The table includes the number of fish removed from statistical analyses for mortalities or later histological analyses. Sample sizes were used for statistical analysis of incision openness, ulceration, and redness.

Day		6-Point	Wide "N"	Wide "N" Knot	Second Skin	Total
Day 7	Mortality	2	1	2	1	6
	Histology	0	0	0	0	0
	N	**63**	**66**	**64**	**65**	**258**
Day 14	Mortality	0	1	0	0	1
	Histology	2	2	2	2	8
	N	**61**	**63**	**62**	**63**	**249**
Day 21	Mortality	0	0	0	0	0
	Histology	2	2	2	2	8
	N	**59**	**61**	**60**	**61**	**241**
Day 28	Mortality	0	0	0	0	0
	Histology	1	1	1	1	4
	N	**58**	**60**	**59**	**60**	**237**

Table 2.3. Sample Sizes for Each Treatment at 17°C by Sample Day. The table includes the number of fish removed from statistical analyses for mortalities or later histological analyses. Sample sizes were used for statistical analysis of incision openness, ulceration, and redness.

Day		6-Point	Wide "N"	Wide "N" Knot	Second Skin	Total
Day 7	Mortality	8	5	7	6	26
	Histology	0	0	0	0	0
	N	**61**	**61**	**64**	**62**	**248**
Day 14	Mortality	10	9	4	9	32
	Histology	1	2	2	1	6
	N	**49**	**49**	**58**	**53**	**209**
Day 21	Mortality	14	9	14	11	48
	Histology	1	3	2	2	8
	N	**34**	**37**	**42**	**39**	**152**
Day 28	Mortality	6	5	6	6	23
	Histology	2	1	2	2	7
	N	**26**	**31**	**34**	**31**	**122**

2.5 Statistical Analysis

Categorical covariates included four suture treatments (6-Point, Wide "N", Wide "N" Knot, and Second Skin), four exam days (Day 7, Day 14, Day 21, and Day 28), and two holding temperatures (12 and 17°C). 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 Chi Squared Test (χ^2) 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 χ^2 and ANOVA were used.

3.0 Results

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

3.1 Fish Size

For all SYC, FL was a significant predictor of WW (N = 580, $F(1, 578) = 2708.48$, $P < 0.0001$). The linear relationship between FL and WW can be described as WW = -33.44679 + 0.4476421*FL (R^2 = 0.83; Figure 3.1). The SYC FL ranged from 96 to 117 mm ($\bar{x}$ =107.8 ± 4.8) and the WW ranged from 9.3 to 21.3 ($\bar{x}$ = 14.8 ± 2.4 g). The WW was significantly higher for fish in the 12°C treatment compared to the 17°C treatment (N = 580, $F(1, 578) = 115.83$, $P < 0.0001$). WW did not significantly vary with suture treatment in the 12°C treatment (N = 284, $F(4, 279) = 1.82$, $P = 0.1247$; Figure 3.2) or 17°C treatment (N = 296, $F(4, 291) = 0.30$, $P = 0.8758$; Figure 3.3), so fish could be pooled for the following analyses.

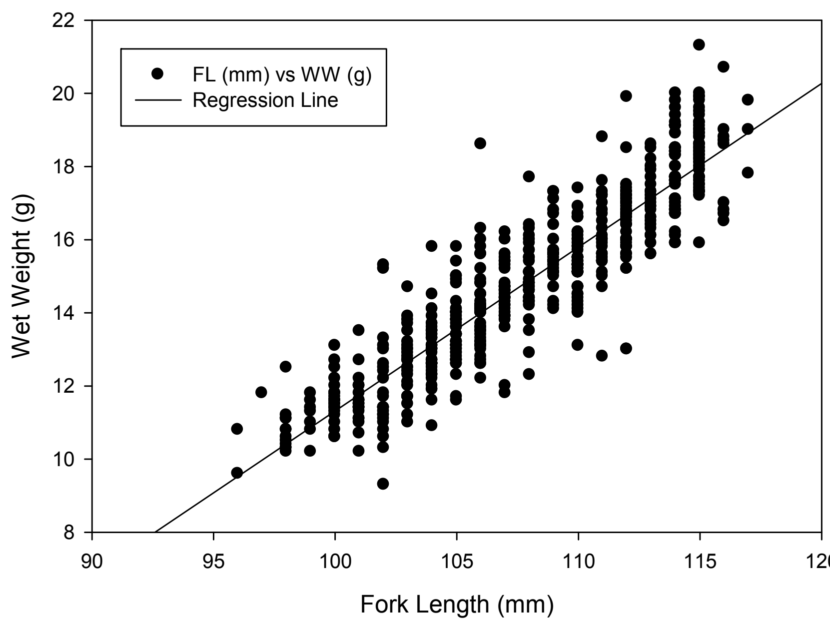

Figure 3.1. Wet Weights (g) by Fork Lengths (mm) of all Study Fish. Each filled circle (●) represents an individual fish at the beginning of the study.

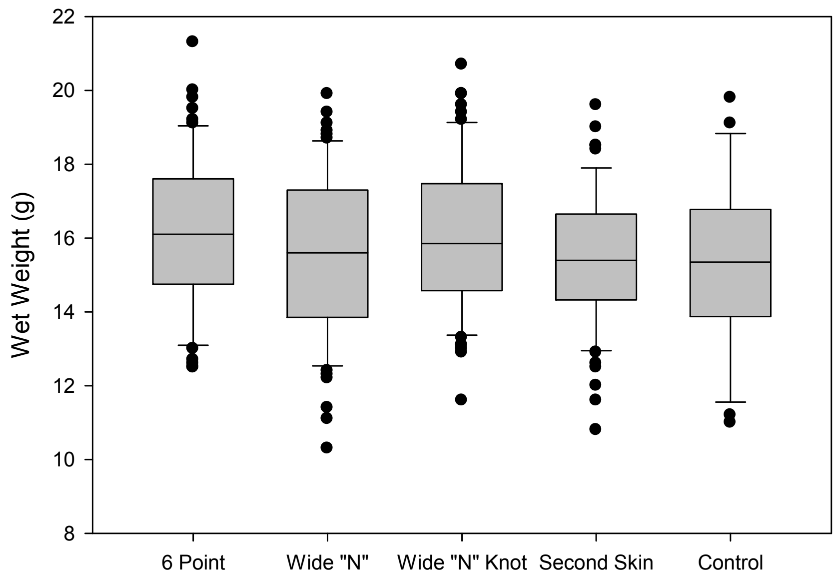

Figure 3.2. Wet Weights (g) of Study Fish for Each Treatment at 12°C. Each box represents the median and upper and lower quartiles for study fish WW at the beginning of the experiment. The whiskers represent the 5th and 95th percentiles and the "•" indicate points that are outside of the reaminder of the data.

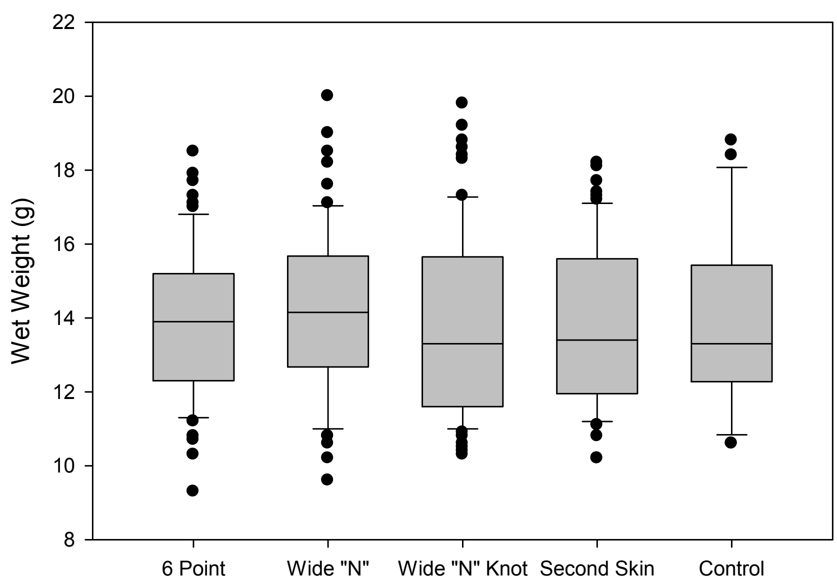

Figure 3.3. Wet Weights (g) of Study Fish for Each Treatment at 17°C. Each box represents the median and upper and lower quartiles for study fish WW at the beginning of the experiment. The whiskers represent the 5th and 95th percentiles and the "•" indicate points that are outside of the reaminder of the data.

3.2 Mortalities

To address whether suture pattern and type influenced mortality rates, we examined the mortality frequency among treatment groups (Table 3.1). Overall experimental mortality was low (3.0%) for fish held at 12°C and did not significantly vary between treatment groups (N = 266, χ^2 = 0.68, P = 0.9552; Table 3.1). Mortalities for fish held at 12°C occurred from 1 to 14 days post-surgery (median = 1 day post-surgery). At 17°C, overall experimental mortality was relatively high (48.5%) and significantly varied between treatment groups (N = 274, χ^2 = 11.96, P = 0.0177; Table 3.1); however, this relationship was mainly driven by the Control group and when the Control group was removed from analysis, mortality did not vary between treatment groups (N = 252, χ^2 = 2.411621; P = *0*.4915). At 17°C, the 6-Point treatment group suffered the highest mortality rate over the range of the study, followed by the Second Skin, Wide "N" Knot, Wide "N", and Control treatment groups. Mortalities occurred from 0 to 27 days post-surgery (Median = 15 days post-surgery).

Table 3.1. Mortality Frequency for Fish in Each Treatment Group. Frequency of occurrence as a percentage is shown in parentheses for each treatment.

Temperature	Mortality	6-Point	Wide "N"	Wide "N" Knot	Second Skin	Control
12°C	No	58	60	59	60	21
	Yes	2 (3.3%)	2 (3.2%)	2 (3.3)%	1 (1.6%)	1 (4.5%)
17°C	No	26	31	34	32	18
	Yes	38 (59.4%)	28 (47.5%)	31 (47.7%)	32 (50.0%)	4 (18.2%)

3.3 Tag Loss

To address whether one suture pattern and type had a greater rate of tag loss, we analyzed the number of dropped AT and PIT tags. At 12°C, no ATs or PITs were dropped; therefore, statistical analyses were not performed. At 17°C, AT loss was relatively high (8.1%) and a total of 10 ATs were dropped by Day 28. Five fish in the Second Skin treatment group, three fish in the Wide "N" treatment group, one fish in the Wide "N" Knot treatment group, and one fish in the 6-Point treatment group dropped ATs between days 14 and 28 (Median = Day 28). The frequency of dropped ATs was not significantly different between treatment groups at 17°C (N = 123, χ^2 = 4.41, P = 0.2208; Table 3.2). At 17°C, there were no dropped PITs in live fish; however dropped PITs occurred in dead fish, but these were not factored into the frequency of tag loss.

Table 3.2. AT Retention for Each Treatment. Frequency of occurrence as a percentage is shown in parentheses for each treatment group.

Temperature	Tag Retention	6-Point	Wide "N"	Wide "N" Knot	Second Skin
12°C	Not Dropped	58	60	59	60
	Dropped	0	0	0	0
17°C	Not Dropped	25	28	33	27
	Dropped	1 (3.8%)	3 (9.7%)	1 (2.9%)	5 (15.6%)

3.4 Incision Openness

To determine whether one suture pattern or type had a greater influence on tag loss or physiological stress, we examined incision openness (surface area; mm^2) on days 7, 14, 21, and 28 (Table 3.3). On Day 7 at 12°C, the 6-Point, Wide "N", and Wide "N" Knot treatment groups had fish with incision openness; the greatest average openness occurred in the Wide "N" treatment group (range = 0 to 3.82 mm^2; Table 3.3). Incision openness significantly varied with treatments (N = 258, $\chi^2 = 13.8356$, $P = 0.0031$; Figure 3.4, Figure 3.5). Post hoc analyses revealed that the Wide "N" treatment group had significantly more incision openness than the Second Skin treatment group. On Day 7 at 17°C, all treatment groups had fish with incision openness; the greatest average openness occurred in the Wide "N" treatment group (range = 0 to 11.29 mm^2). Incision openness significantly varied by treatment (N = 246, $\chi^2 = 32.5104$, $P < 0.0001$; Figure 3.6, Figure 3.7). Post hoc analyses revealed that fish in the Wide "N" treatment group had significantly greater incision openness than those in the 6-Point, Wide "N" Knot, and Second Skin treatment groups (all $P < 0.05$).

On Day 14 at 12°C, the 6-Point, Wide "N", and Wide "N" Knot treatment groups had fish with incision openness; the greatest average openness occurred in the Wide "N" treatment group (range = 0 to 5.13 mm^2; Table 3.2). At 12°C, incision openness significantly varied between treatment groups (N = 249, $\chi^2 = 8.5494$; $P = 0.0359$; Figure 3.4), but post hoc analyses revealed no differences between them. On Day 14 at 17°C, all treatment groups had fish with incision openness; the greatest average openness occurred in the Wide "N" treatment group (range = 0 to 10.25 mm^2). At 17°C, incision openness did not vary by treatment group (N = 209, $\chi^2 = 0.4815$, $P = 0.9229$; Figure 3.6).

On Day 21 at 12°C, the Second Skin treatment group was the only group to have fish with incision openness (range = 0 to 1.42 mm^2; Table 3.2). On Day 21 at 17°C, all treatments had fish with incision openness; the greatest average incision openness occurred in the Wide "N" treatment group (range = 0 to 11.22 mm^2). Incision openness did not vary by treatment group at 12°C (N = 241, $\chi^2 = 2.9508$, $P = 0.3993$; Figure 3.4) or 17°C (N = 152, $\chi^2 = 3.1135$, $P = 0.3745$; Figure 3.6).

On Day 28 at 12°C, the Second Skin treatment group was the only one that had fish with incision openness (range 0 to 1.95 mm^2; Table 3.2). Incision openness did not vary by treatment group for fish at 12°C (N = 237, $\chi^2 = 2.9500$, $P = 0.3994$; Figure 3.4). On Day 28 at 17°C, all treatment groups had fish with incision openness; the greatest average openness occurred in the Second Skin treatment group (range = 0 to 7.59 mm^2), but incision openness did not significantly vary by treatment group (N = 122, $\chi^2 = 5.7495$, $P = 0.1245$; Figure 3.6).

Table 3.3. Incision Openness (mm^2) on Days 7, 14, 21, and 28 by Suture Treatment Group and Holding Temperature. Average incision openness ± SD and frequency of occurrence as a percentage is shown in parentheses for each treatment.

Temperature	Observation Day	6-Point	Wide "N"	Wide "N" Knot	Second Skin
12°C	7	0.05 ± 0.37 (1.6%)	0.21 ± 0.68 (12.1%)	0.09 ± 0.53 (3.1%)	0.0 (0%)
	14	0.01 ± 0.10 (1.6%)	0.21 ± 0.82 (9.4%)	0.03 ± 0.26 (1.6%)	0.0 (0%)
	21	0.0 (0%)	0.0 (0%)	0.0 (0%)	1.42 ± 0.0 (1.6%)
	28	0.0 (0%)	0.0 (0%)	0.0 (0%)	0.03 ± 0.25 (1.6%)
17°C	7	0.02 ± 0.18 (1.6%)	1.25 ± 2.43 (29.5%)	0.31 ± 1.16 (7.8%)	0.56 ± 4.40 (3.2%)
	14	0.08 ± 0.32 (6.1%)	0.26 ± 1.50 (4.1%)	0.09 ± 0.46 (3.4%)	0.15 ± 0.76 (5.7%)
	21	0.05 ± 0.29 (2.9%)	0.37 ± 1.88 (5.4%)	0.01 ± 0.09 (2.4%)	0.24 ± 0.75 (10.3%)
	28	0.09 ± 0.44 (7.7%)	0.63 ± 1.91 (16.1%)	0.08 ± 0.32 (5.9%)	1.14 ± 2.31 (22.9%)

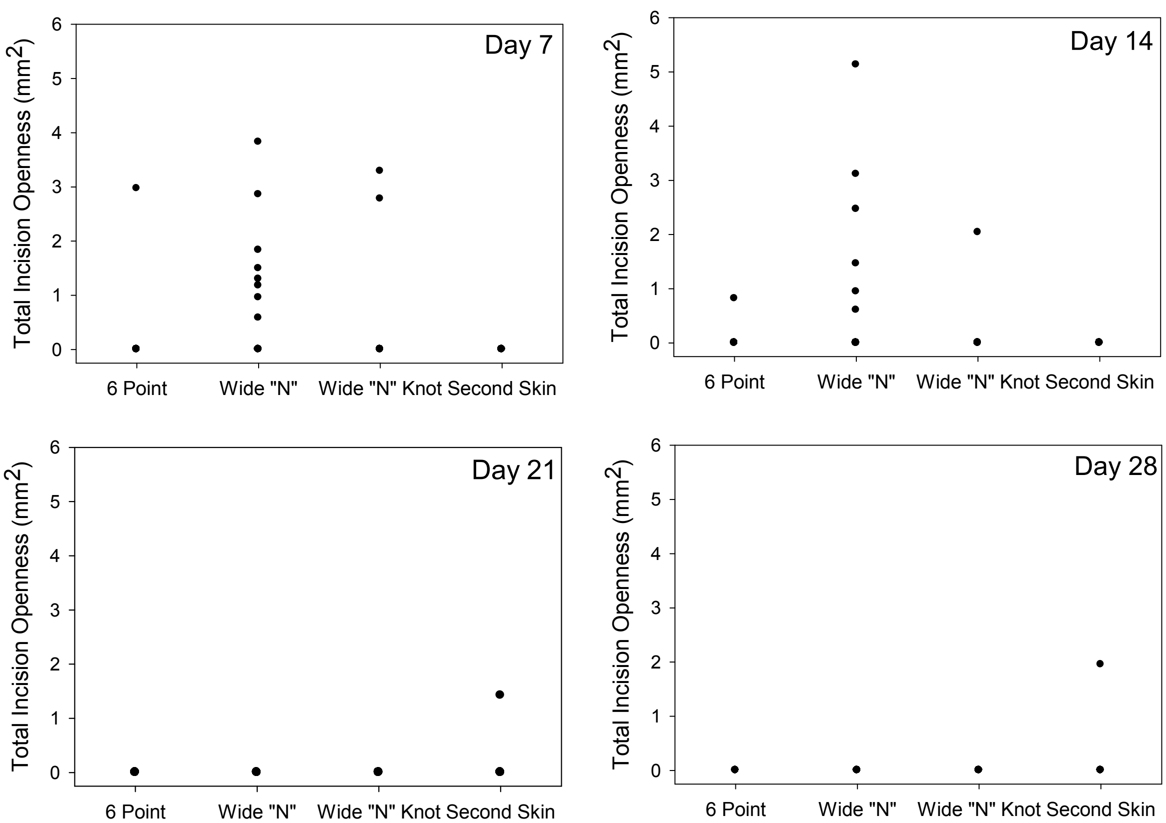

Figure 3.4. Total Incision Openness (mm^2) by Treatment at 7, 14, 21, and 28 Days Post-Surgery for Fish Held at 12°C. Data points overlap at 0.00 mm^2.

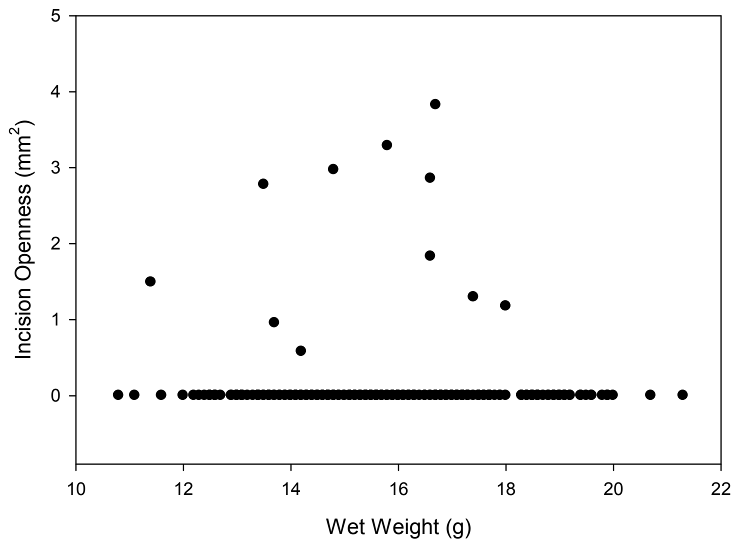

Figure 3.5. Total Incision Openness (mm^2) Plotted Against the Wet Weight of Individual Fish, Day 7 at 12°C. WW was likely not a determinative factor in the incision openness. Data points only overlapped at 0.00 mm^2.

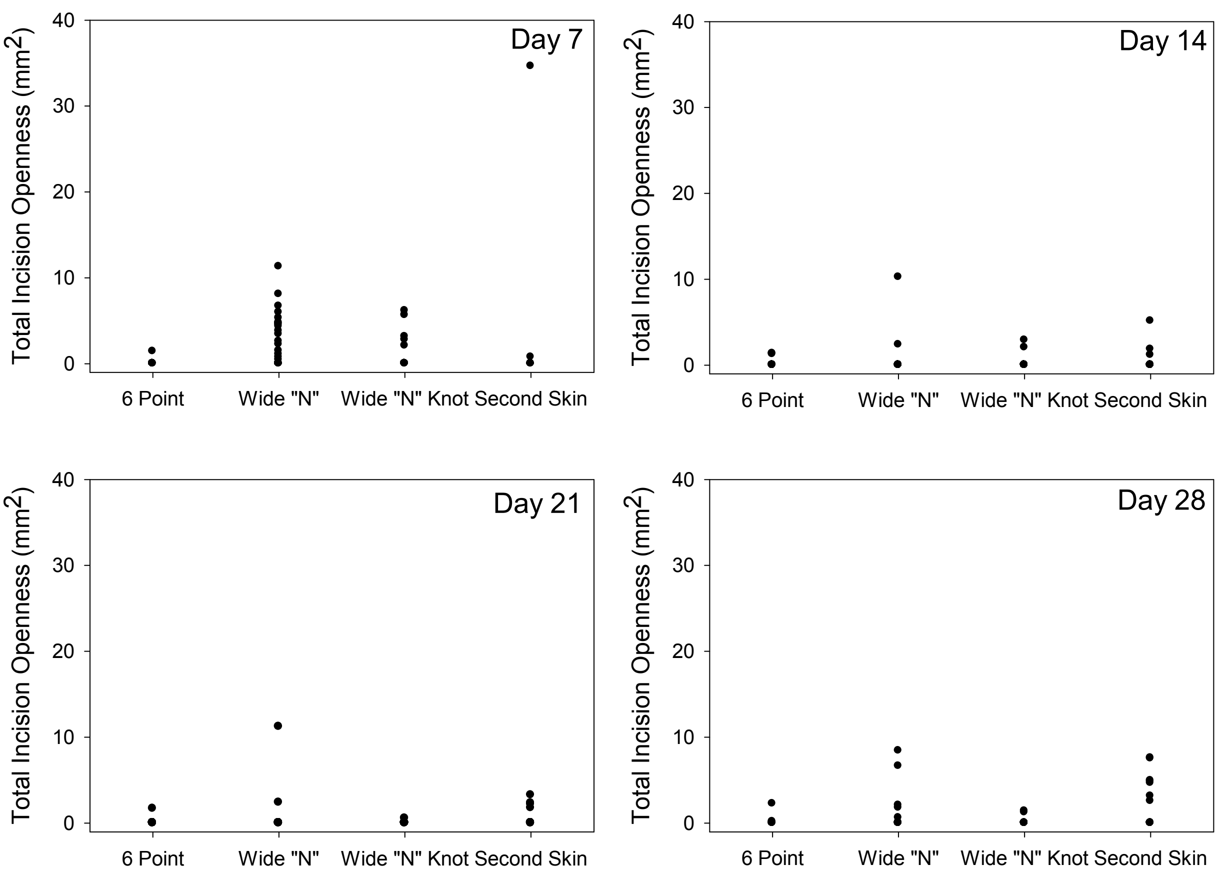

Figure 3.6. Total Incision Openness (mm^2) by Treatment at 7, 14, 21, and 28 Days Post-Surgery for Fish Held at 17°C. Data points only overlapped at 0.00 mm^2.

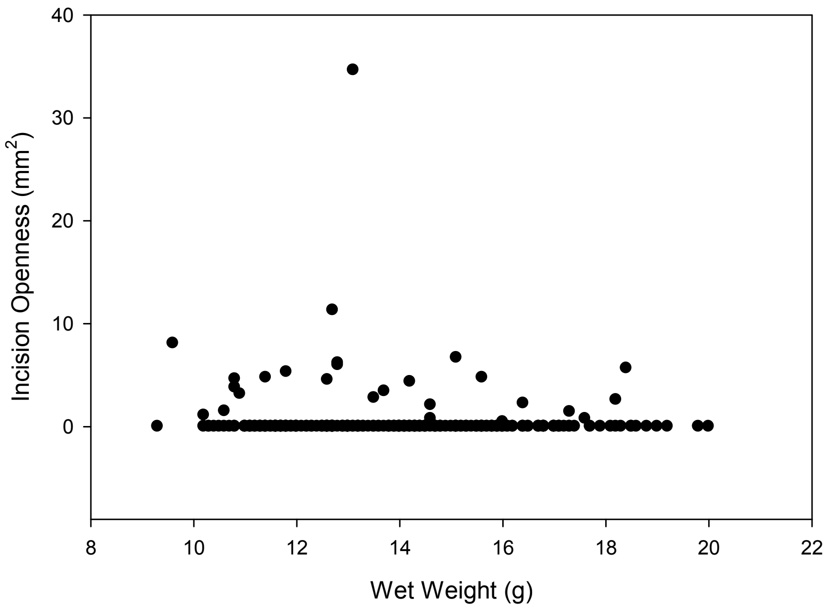

Figure 3.7. Total Incision Openness (mm^2) Plotted Against the Wet Weight of Individual Fish, Day 7 at 17°C. WW was likely not a determinative factor in the incision openness. Data points only overlapped at 0.00 mm^2.

3.5 Functional Suture

We examined sutures to determine if one suture pattern and its associated needle type resulted in more functional sutures (i.e., present and maintained proper tension) than others at Site 1, Site 2, and if applicable, Site 3 (Figure 2.1 and Table 3.4 and Table 3.5). At Site 1 on Day 7 at 12°C, the Wide "N" treatment group had the fewest functional sutures (77.8%), followed by the 6-Point (81.3%), Wide "N" Knot (100%), and Second Skin (100%) treatment groups (N = 209, χ^2 = 33.42, P < 0.001; Table 3.4). At 17°C, the Wide "N" treatment group had the fewest functional sutures (86.7%), followed by the 6-Point (91.6%), Wide "N" Knot (96.7%), and Second Skin (100%) treatment groups (N = 192, χ^2 = 9.87, P = 0.0197; Table 3.5).

At Site 1 on Day 14 at 12°C, the Wide "N" treatment group had the fewest functional sutures (66.6%), followed by the 6-Point (81.1%), Wide "N" Knot (98.1%), and Second Skin (100%) treatment groups (N = 189, χ^2 = 30.89, P < 0.0001; Table 3.4). At 17°C, the 6-Point treatment group had the fewest functional sutures (71.9%), followed by the Wide "N" (82.4%), Wide "N" Knot (96.0%), and Second Skin (97.4%) treatment groups (N = 138, χ^2 = 14.92, P = 0.0019; Table 3.5).

At Site 1 on Day 21 at 12°C, the Wide "N" treatment group had the fewest functional sutures (50.0%), followed by the 6-Point (67.4%), Wide "N" Knot (100%), and Second Skin (100%) treatment groups (N = 173, χ^2 = 57.32, P < 0.0001; Table 3.4). At 17°C, the 6-Point treatment group had the fewest functional sutures (56.3%), followed by the Wide "N" (87.5%), Second Skin (88.9%), and Wide "N" Knot (93.8%) treatment groups (N = 83, χ^2 = 10.29, P = 0.0162; Table 3.5).

At Site 1 on Day 28 at 12°C, the 6-Point treatment group had the fewest functional sutures (73.5%), followed by the Wide "N" (83.3%), Wide "N" Knot (93.2%), and Second Skin (98.3%) treatment groups

(N = 143, χ^2 = 14.90, P = 0.0019; Table 3.4). At 17°C, the 6-Point treatment group had the fewest functional sutures (28.6%), followed by the Wide "N" (33.3%), Wide "N" Knot (57.9%), and Second Skin (88.9%) treatment groups (N = 47, χ^2 = 10.90, P = 0.0123; Table 3.5).

Table 3.4. Number of Functional Sutures by Entry/Exit Point for Each Treatment at 12°C. 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 Point	6-Point	Wide "N"	Wide "N" Knot	Second Skin
7	1	Yes	39	28	60	65
	1	No	9	8	0	0
	2	Yes	56	23	32	65
	2	No	0	13	12	0
	3	Yes	34	NA	NA	NA
	3	No	12	NA	NA	NA
14	1	Yes	43	14	52	62
	1	No	10	7	1	0
	2	Yes	50	15	29	63
	2	No	4	6	11	0
	3	Yes	37	NA	NA	NA
	3	No	16	NA	NA	NA
21	1	Yes	29	10	50	60
	1	No	14	10	0	0
	2	Yes	40	7	22	59
	2	No	3	9	11	1
	3	Yes	22	NA	NA	NA
	3	No	10	NA	NA	NA
28	1	Yes	25	5	41	58
	1	No	9	1	3	1
	2	Yes	33	5	20	58
	2	No	4	1	6	2
	3	Yes	15	NA	NA	NA
	3	No	12	NA	NA	NA
Necropsy	Suture present		1	0	1	0
	Suture not present		24	48	16	1

NA = Not Applicable.

At Site 2 on Day 7 at 12°C, the Wide "N" treatment group had the fewest functional sutures (63.9%), followed by the Wide "N" Knot (72.7%), 6-Point (100%) and Second Skin (100%) treatment groups (N = 201, χ^2 = 52.32, P < 0.0001; Table 3.4). At 17°C, the Wide "N" treatment group had the fewest functional sutures (77.8%), followed by the Wide "N" Knot (88.1%), 6-Point (100%), and Second Skin (100%) treatment groups (N = 178, χ^2 = 23.29, P < 0.0001; Table 3.5).

Table 3.5. Number of Functional Sutures by Entry/Exit Point for Each Treatment at 17°C. The last two rows indicate whether the suture was seen internally either in the body cavity or embedded in tissue.

Observation Day	Site	Functional at Entry/Exit Point	6-Point	Wide "N"	Wide "N" Knot	Second Skin
7	1	Yes	44	26	59	53
	1	No	4	4	2	0
	2	Yes	54	21	37	55
	2	No	0	6	5	0
	3	Yes	29	NA	NA	NA
	3	No	14	NA	NA	NA
14	1	Yes	23	14	48	38
	1	No	9	3	2	1
	2	Yes	33	9	25	42
	2	No	1	5	2	0
	3	Yes	22	NA	NA	NA
	3	No	1	NA	NA	NA
21	1	Yes	9	7	30	24
	1	No	7	1	2	3
	2	Yes	14	3	8	25
	2	No	2	3	6	5
	3	Yes	7	NA	NA	NA
	3	No	5	NA	NA	NA
28	1	Yes	2	1	11	16
	1	No	5	2	8	2
	2	Yes	7	0	0	15
	2	No	2	3	9	6
	3	Yes	3	NA	NA	NA
	3	No	2	NA	NA	NA
Necropsy	Suture present		0	1	1	0
	Suture not present		16	21	19	4

NA = Not Applicable.

At Site 2 on Day 14 at 12°C, the Wide "N" treatment group had the fewest functional sutures (71.4%), followed the Wide "N" Knot (72.5%), 6-Point (92.6%), and the Second Skin (100%) treatment groups (N = 178, $\chi^2 = 28.49$, $P < 0.0001$; Table 3.4). At 17°C, the Wide "N" group had the fewest functional sutures (64.3%), followed by the Wide "N" Knot (92.6%), 6-Point (97.1%), and Second Skin (100%) treatment groups (N = 117, $\chi^2 = 16.83$, $P = 0.0008$; Table 3.5).

At Site 2 on Day 21 at 12°C, the Wide "N" treatment group had the fewest functional sutures (43.8%), followed by the Wide "N" Knot (66.7%), 6-Point (93.0%), and Second Skin (98.3%) treatment groups (N = 152, $\chi^2 = 36.72$, $P < 0.001$; Table 3.4). At 17°C, although not significantly different, the

Wide "N" treatment group had the fewest functional sutures (50.0%), followed by the Wide "N" Knot (57.1%), Second Skin (83.3%), and 6-Point (87.5%) treatment groups (N = 66, χ^2 = 6.58, P = 0.0866; Table 3.5).

At Site 2 on Day 28 at 12°C, the Wide "N" Knot treatment group had the fewest functional sutures (76.9%), followed by the Wide "N" (83.3%), 6-Point (89.2%), and Second Skin (96.7%) treatment groups (N = 129, χ^2 = 7.93, P = 0.0475; Table 3.4). At 17°C, the Wide "N" and Wide "N" Knots had no remaining functional sutures, followed by the Second Skin (71.4%) and 6-Point (77.8%) treatment groups (N = 42, χ^2 = 23.47, P < 0.0001; Table 3.5).

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 3.4 and Table 3.5).

3.6 Ulceration and Redness

To determine whether suture pattern or type influenced tag loss or physiological stress, we examined the frequency of ulceration and redness occurrences and the total surface area by treatment (Figure 3.8). Simplifying the analysis to presence or absence of ulcerations, on Day 7 at 12°C there was no significant difference in the frequency (N= 258, χ^2 = 2.97, P = 0.3962) or the total surface area of ulceration around the incision and/or the suture entry/exit sites (surface area measurements, mm^2) between treatment groups (N = 258, $F(3, 254)$ = 0.77, P = 0.5111; Table 3.6, Figure 3.9). At 17°C, there was no significant difference in the frequency (N = 247, χ^2 = 1.14, P = 0.7670) or the total surface area of ulceration between treatment groups (N = 247, $F(3, 243)$ = 1.0146, P = 0.3868 Table 3.7, Figure 3.10).

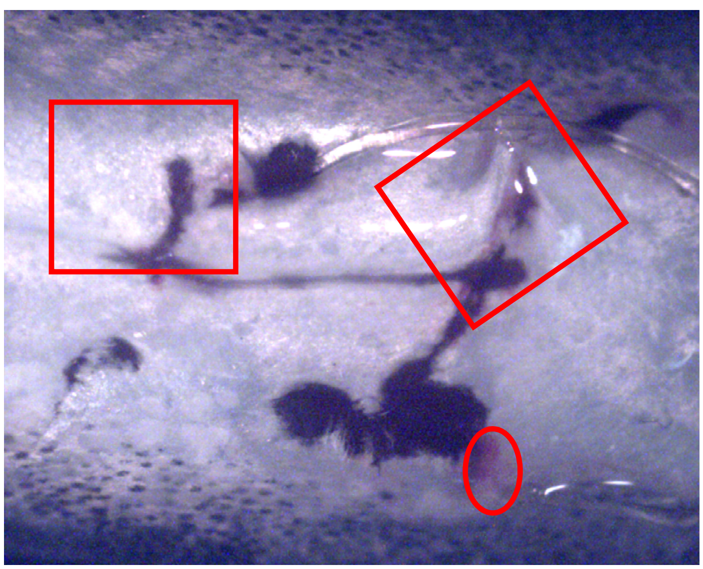

Figure 3.8. Example of Ulceration and Redness Caused by Suture Tearing. The red circle (○) highlights redness not directly incorporated with ulceration. The two red squares (□) denote ulceration and redness that were separated using Image J. The circle and square do not denote the actual Image J patterns and measurements used for the final summations of total ulceration and redness.

Table 3.6. Frequency and Mean Area of Ulceration for Each Treatment for Fish Held at 12°C. 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	Second Skin
7	Yes	4 (6.3%)	2 (3.0%)	1 (1.6%)	1 (1.5%)
	No	59	64	63	64
	$\bar{x}$ mm^2 ± SD	0.02 ± 0.09	0.01 ± 0.06	0.00 ± 0.03	0.01± 0.06
14	Yes	28 (45.9%)	10 (15.9%)	10 (16.1%)	15 (23.8%)
	No	33	53	52	48
	$\bar{x}$ mm^2 ± SD	0.29 ± 0.41	0.07 ± 0.21	0.10 ± 0.30	0.08 ± 0.17
21	Yes	20 (33.9%)	6 (9.8%)	18 (30.0%)	3 (4.9%)
	No	39	55	42	58
	$\bar{x}$ mm^2 ± SD	0.36 ± 0.73	0.06 ± 0.21	0.19 ± 0.39	0.03 ± 0.15
28	Yes	15 (25.9%)	2 (3.3%)	11 (18.6%)	5 (8.3%)
	No	43	58	48	55
	$\bar{x}$ mm^2 ± SD	0.55 ± 1.26	0.02 ± 0.10	0.22 ± 0.63	0.05 ± 0.22

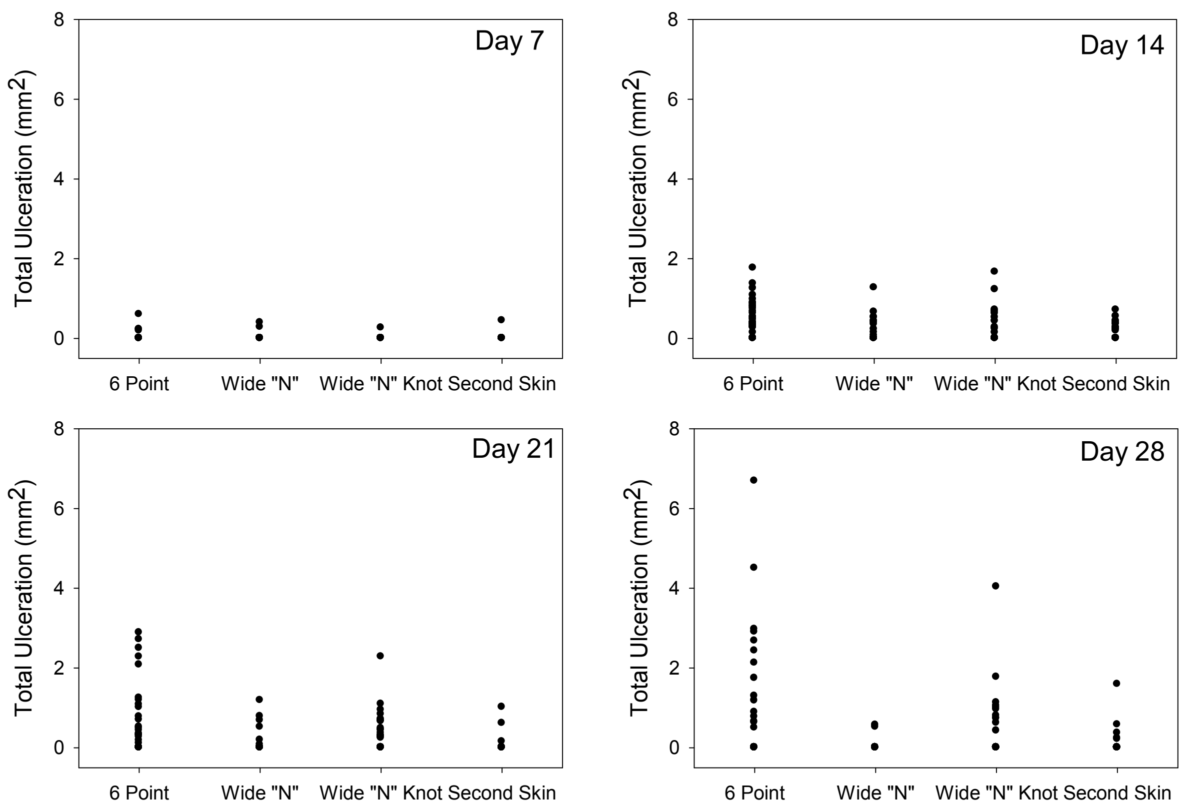

Figure 3.9. Total Ulceration (mm^2) by Treatment for 7, 14, 21, and 28 Days Post-Surgery at 12°C. Data points only overlapped at 0.00 mm^2.

Table 3.7. Frequency and Mean Area of Ulceration for Each Treatment for Fish Held at 17°C. 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	Second Skin
	Yes	15 (24.6%)	19 (31.1%)	21 (32.8%)	19 (30.6%)
7	No	46	42	43	43
	$\bar{x}$ mm^2 ± SD	0.21 ± 0.51	0.14 ± 0.24	0.62 ± 3.27	0.49 ± 1.14
	Yes	28 (57.1%)	12 (24.5%)	34 (58.6%)	20 (37.7%)
14	No	21	37	24	33
	$\bar{x}$ mm^2 ± SD	1.41 ± 1.74	0.45 ± 1.04	0.85 ± 1.14	1.06 ± 2.23
	Yes	17 (50.0%)	8 (21.6%)	24 (57.1%)	12 (30.8%)
21	No	17	29	18	27
	$\bar{x}$ mm^2 ± SD	1.86 ± 3.22	0.44 ± 1.32	0.92 ± 1.10	0.83 ± 1.87
	Yes	9 (34.6%)	3 (9.7%)	15 (44.1%)	7 (22.6%)
28	No	17	28	19	24
	$\bar{x}$ mm^2 ± SD	1.51 ± 3.50	0.40 ± 1.71	0.81 ± 1.07	1.25 ± 2.93

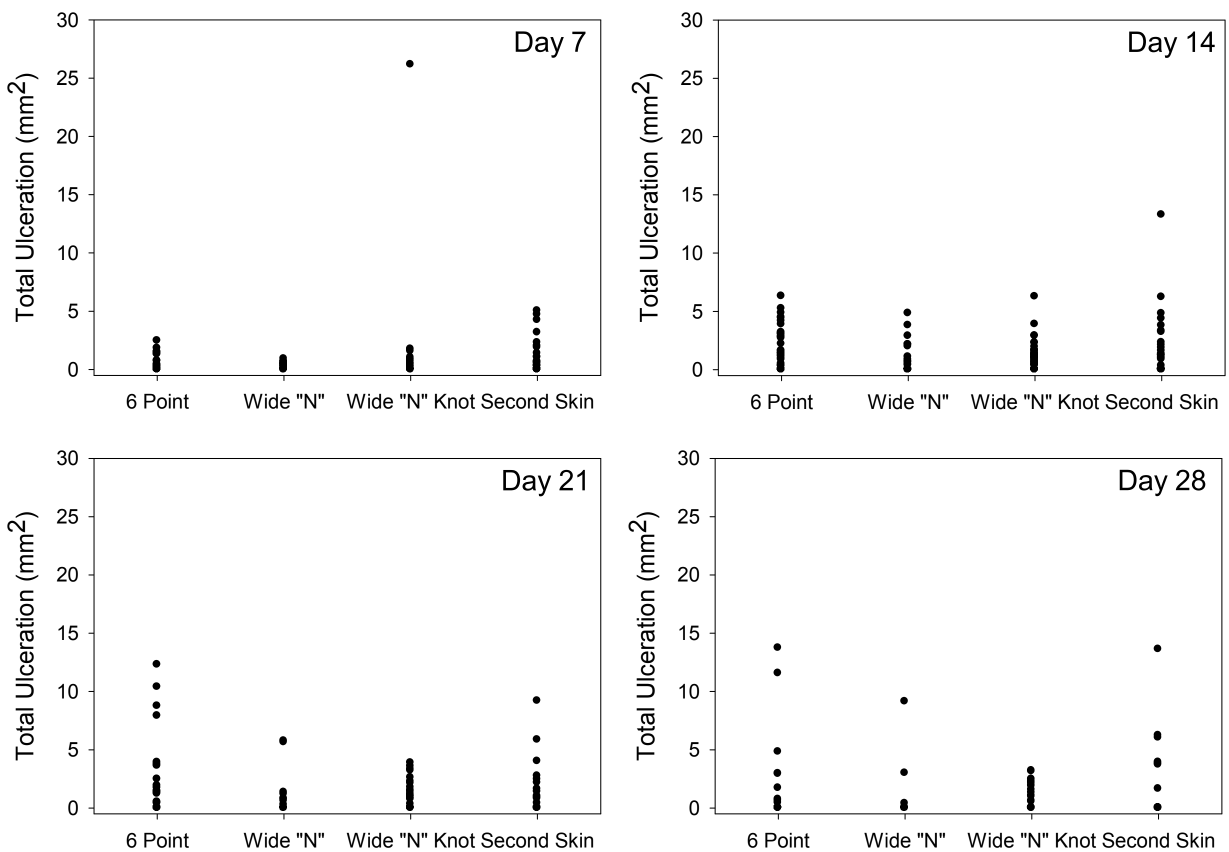

Figure 3.10. Total Ulceration (mm^2) by Treatment for 7, 14, 21, and 28 Days Post-Surgery at 17°C. Data points only overlapped at 0.00 mm^2.

On Day 14 at 12°C, the 6-Point treatment had significantly greater frequency of ulceration (N= 249, χ^2 = 18.45, P = 0.0004), followed by the Second Skin, Wide "N" Knot, and Wide "N" treatments. The 6-Point treatment group also had significantly greater total ulceration surface area than the Wide "N", Wide "N" Knot, and Second Skin treatment groups (N = 249, $F(3, 245) = 7.66$, $P < 0.0001$; Table 3.6, Figure 3.9). At 17°C, the Wide "N" Knot treatment group had significantly greater frequency of ulceration (N = 209, χ^2 = 17.22, P = 0.0006), followed by the 6-Point, Second Skin, and Wide "N" treatment groups. The 6-Point treatment group had significantly greater total ulceration surface area than the Wide "N" treatment group (N = 209, $F(3, 205) = 3.05$, P = 0.0295; Table 3.7, Figure 3.10).

On Day 21 at 12°C, the 6-Point treatment group had significantly greater frequency of ulceration (N = 241, χ^2 = 25.82, $P < 0.0001$), followed by the Wide "N" Knot, Wide "N", and Second Skin. The 6-Point treatment group also had significantly greater total ulceration than the Wide "N" and Second Skin treatment groups (N = 241, $F(3, 237) = 7.52$, $P < 0.0001$; ANOVA; Table 3.6, Figure 3.9). At 17°C, the Wide "N" Knot treatment group had significantly greater frequency of ulceration (N= 152, χ^2 = 13.48, P = 0.0037), followed by the 6-Point, Second Skin, and Wide "N" treatment groups. The 6-Point treatment had significantly greater total ulceration surface area than the Wide "N" treatment group (N = 152, $F(3, 148) = 3.20$, P = 0.025; ANOVA; Table 3.7, Figure 3.10).

On Day 28 at 12°C, the 6-Point treatment group had significantly greater frequency of ulceration (N = 237, χ^2 = 16.27, P = 0.001), followed by the Wide "N" Knot, Second Skin, and Wide "N" treatment groups. The 6-Point treatment group also had significantly more total ulceration surface area than the Wide "N" treatment group (N = 237, $F(3, 233) = 3.50$, P = 0.0002; ANOVA; Table 3.6, Figure 3.9). At 17°C, the Wide "N" Knot treatment group had significantly greater frequency of ulceration (N= 122, χ^2 = 11.34, P = 0.01), followed by the 6-Point, Second Skin, and Wide "N" treatment groups. However, total ulceration surface area was not different between treatment groups (N = 122, $F(3, 233) = 1.20$, P = 0.3112; ANOVA; Table 3.7, Figure 3.10).

Table 3.8. Frequency and Mean Area of Redness for Each Treatment at 12°C

Observation Day	Redness	6-Point	Wide "N"	Wide "N" Knot	Second Skin
	Yes	28 (44.4%)	20 (30.3%)	30 (46.9%)	13 (20.0%)
7	No	35	46	34	52
	$\bar{x}$ mm^2 ± SD	0.60 ± 1.34	0.24 ± 0.54	0.49 ± 1.53	0.10 ± 0.38
	Yes	20 (32.9%)	14 (22.2%)	13 (30.0%)	8 (12.7%)
14	No	41	49	49	55
	$\bar{x}$ mm^2 ± SD	0.16 ± 0.34	0.08 ± 0.21	0.14 ± 0.35	0.04 ± 0.13
	Yes	16 (27.1%)	5 (8.2%)	6 (10.0%)	6 (9.8%)
21	No	43	56	54	55
	$\bar{x}$ mm^2 ± SD	0.12 ± 0.39	0.04 ± 0.16	0.05 ± 0.21	0.04 ± 0.18
	Yes	8 (13.8%)	4 (6.7%)	7 (11.9%)	3 (5.1%)
28	No	50	56	52	57
	$\bar{x}$ mm^2 ± SD	0.04 ± 0.12	0.02 ± 0.11	0.05 ± 0.25	0.02 ± 0.09

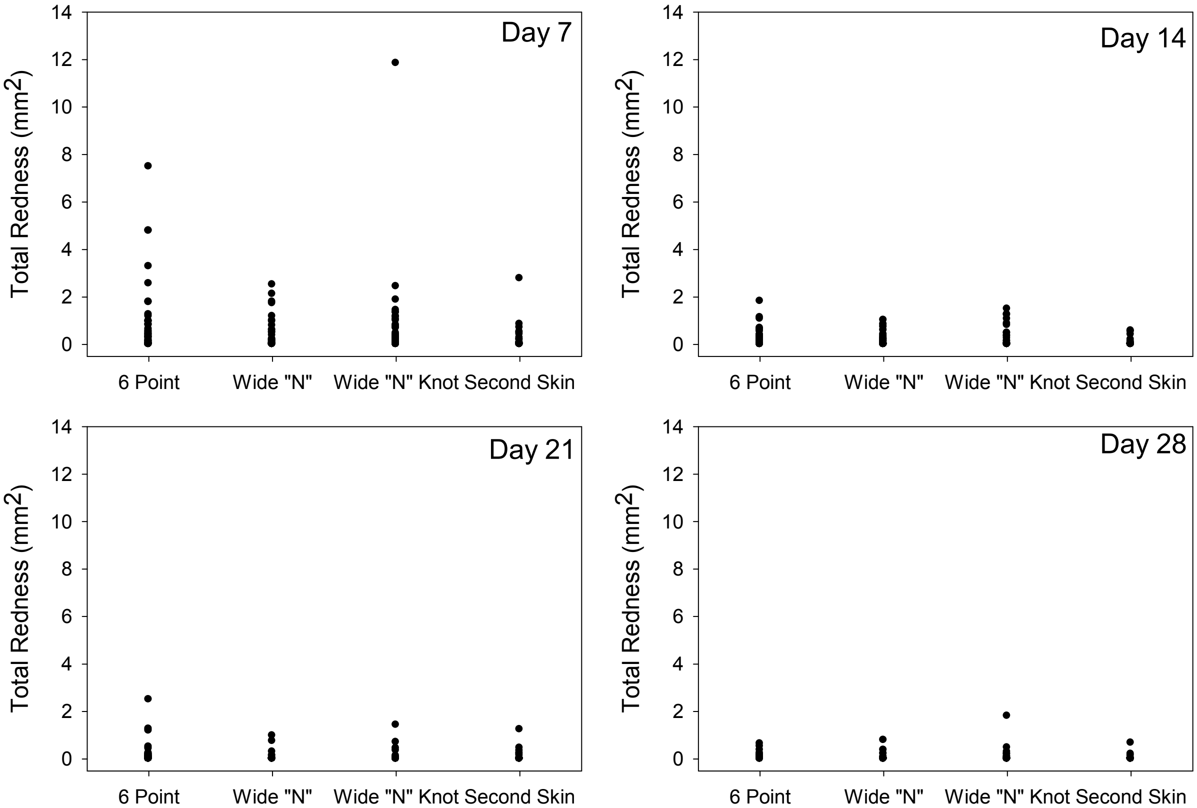

Figure 3.11. Total Redness (mm^2) by Treatment for 7, 14, 21, and 28 Days Post-Surgery at 12°C. Data points only overlapped at 0.00 mm^2.

Table 3.9. Frequency and Mean Area of Redness for Each Treatment at 17°C

Observation Day	Redness	6-Point	Wide "N"	Wide "N" Knot	Second Skin
7	Yes	28 (45.9%)	17 (27.9%)	25 (39.1%)	26 (41.9%)
	No	33	44	39	36
	$\bar{x}$ mm^2 ± SD	0.34 ± 0.71	0.17 ± 0.48	0.24 ± 0.52	0.66 ± 1.30
14	Yes	9 (18.4%)	4 (8.2%)	14 (24.1%)	27 (50.9%)
	No	40	45	44	26
	$\bar{x}$ mm^2 ± SD	0.12 ± 0.34	0.09 ± 0.50	0.21 ± 0.69	1.72 ± 3.43
21	Yes	5 (14.7%)	3 (8.1%)	9 (21.4%)	6 (15.4%)
	No	29	34	33	33
	$\bar{x}$ mm^2 ± SD	0.09 ± 0.24	0.07 ± 0.30	0.12 ± 0.36	0.41 ± 1.36
28	Yes	3 (11.5%)	0 (0%)	3 (8.8%)	5 (16.1%)
	No	23	31	31	26
	$\bar{x}$ mm^2 ± SD	0.08 ± 0.27	0.0	0.07 ± 0.31	0.07 ± 0.27

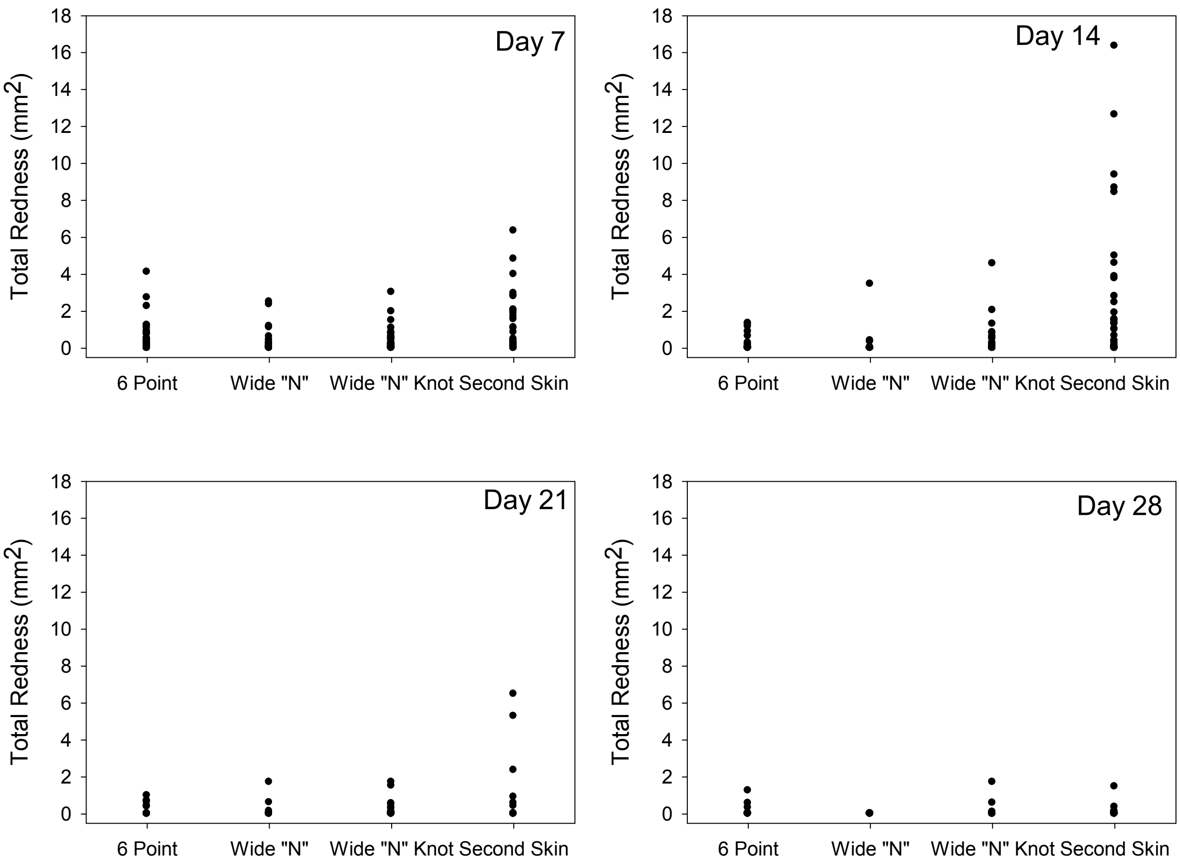

Figure 3.12. Total Redness (mm^2) by Treatment for 7, 14, 21, and 28 Days Post-Surgery at 17°C. Data points only overlapped at 0.00 mm^2.

When simplifying the analysis to the presence or absence of redness for each fish, on Day 7 at 12°C the Wide "N" Knot treatment group had significantly greater frequency of fish with redness, followed by the 6-Point, Wide "N", and the Second Skin treatment groups (N = 258, χ^2 = 13.89, P = 0.0031). When comparing total redness surface area (mm^2) around the incision and/or the suture entry/exits sites, the 6-Point treatment group had significantly greater redness than the Second Skin (N = 258, F(3, 254) = 2.96, P = 0.0330; Table 3.8, Figure 3.11). At 17°C, there was no significant difference in frequency of redness between the treatment groups (N = 248, χ^2 = 4.74, P = 0.1920). However, the Second Skin treatment group had significantly greater total redness surface area than the Wide "N" and Wide "N" Knot treatment groups (N= 248, F(3, 244) = 4.36, P = 0.0052; Table 3.9, Figure 3.12).

On Day 14 at 12°C, there was no significant difference in the frequency of redness between the treatment groups (N = 249, χ^2 = 7.39, P = 0.0604). However, total redness surface area significantly varied between treatments (N = 249, F(3.245) = 2.74, P = 0.0442; Table 3.8, Figure 3.11), but post hoc tests revealed that total redness surface area was similar for the treatment groups (all P > 0.05). At 17°C, the Second Skin group had significantly greater frequency of fish with redness followed by the Wide "N" Knot, 6-Point and Wide "N" treatment groups (N = 209, χ^2 = 26.81, P < 0.0001). The Second Skin treatment group also had significantly greater total redness surface area when compared to the Wide "N", Wide "N" Knot, and 6-Point treatment groups (N = 209, F(3, 205) = 10.31, P < 0.0001; Table 3.9, Figure 3.12).

On Day 21 at 12°C, the 6-Point treatment group had significantly greater frequency of fish with redness, followed by the Wide “N” Knot, Second Skin, and Wide “N” treatment groups (N = 241, χ^2 = 10.70, P = 0.0135). There was no significant difference in the total surface area of redness between the treatment groups (N = 241, $F(3, 237)$ = 1.50, P = 0.2154; Table 3.8, Figure 3.11). At 17°C, there was no difference in the frequency (N = 152, χ^2 = 2.84, P = 0.4162) or total surface area of redness between treatment groups (N = 152, $F(3, 148)$ = 1.80, P = 0.1490; Table 3.9, Figure 3.12).

On Day 28 at 12°C, there was no significant difference in the frequency (N = 237, χ^2 = 3.75, P = 0.2896) or total surface area of redness between treatment groups (N = 237, $F(3, 233)$ = 0.64, P = 0.5869; Table 3.8, Figure 3.11). At 17°C, there was no significant difference in the frequency (N = 122, χ^2 = 7.63, P = 0.0543) or total surface area of redness between treatment groups (N = 122, $F(3, 118)$ = 0.71, P = 0.5485; Table 3.9, Figure 3.12).

3.7 Transmitter Bulge

The effects of suture patterns and fish size were examined for their influence on tag bulging. The occurrence of transmitter bulging was low in this study and only one fish in the Wide “N” treatment group held at 12°C had transmitter bulging (5.36 mm^2) by Day 28.

3.8 Performance Index

Each characteristic of interest was ranked (1 = best, 4 = worst) to assist with recommendations based on the performance of each pattern (Table 3.10 and Table 3.11). The lowest average score indicates the *best* overall suture performance. At 12°C the Second Skin treatment performed better overall, followed by Wide “N” Knot, 6-Point, and Wide “N”. At 17°C, the Wide “N” treatment performed better overall, followed by Wide “N” Knot, 6-Point and Second Skin.

Table 3.10. Performance Index Based on Rank of Each Measured Treatment Observation at 12°C (1 = best, 4 = worst). The treatment with the lowest overall score is considered to have the overall best performance.

	Treatment			
Measured Observation	6-Point	Wide "N"	Wide "N" Knot	Second Skin
Mortality	3	3	3	1
AT/PITs Dropped	2.5	2.5	2.5	2.5
Percentage of Fish with Gaping, day 7	2	4	3	1
Percentage of Fish with Gaping, day 14	2.5	4	2.5	1
Percentage of Fish with Gaping, day 21	2	2	2	4
Percentage of Fish with Gaping, day 28	2	2	2	4
Functional Suture, site 1, day 7	4	4	1	1
Functional Suture, site 1, day 14	4	3	1.5	1.5
Functional Suture, site 1, day 21	4	4	1	1
Functional Suture, site 1, day 28	4	1.5	3	1.5
Functional Suture, site 2, day 7	1	4	4	1
Functional Suture, site 2, day 14	2.5	2.5	4	1
Functional Suture, site 2, day 21	2	4	3	1
Functional Suture, site 2, day 28	2	3	4	1
Area of Ulceration, day 7	3	3	1	3
Area of Ulceration, day 14	4	2	2	2
Area of Ulceration, day 21	4	1.5	3	1.5
Area of Ulceration, day 28	4	1.5	3	1.5
Area of Redness, day 7	4	2	2	2
Area of Redness, day 14	1	3	2	4
Area of Redness, day 21	1.5	3.5	1.5	3.5
Area of Redness, day 28	1	4	2	3
Tag Bulge	2	4	2	2
Average	2.70	2.96	2.39	1.96

Table 3.11. Performance Index Based on Rank of Each Measured Treatment Observation at 17°C (1 = best, 4 = worst). The treatment with the lowest overall score is considered to have the overall best performance.

	Treatment			
Measured Observation	6-Point	Wide "N"	Wide "N" Knot	Second Skin
Mortality	4	1.5	1.5	3
AT/PITs Dropped	1.5	3	1.5	4
Percentage of Fish with Gaping, day 7	1	4	3	2
Percentage of Fish with Gaping, day 14	3.5	1.5	1.5	3.5
Percentage of Fish with Gaping, day 21	1.5	3	1.5	4
Percentage of Fish with Gaping, day 28	1.5	3	1.5	4
Functional Suture, site 1, day 7	3.5	3.5	2	1
Functional Suture, site 1, day 14	4	3	2	1
Functional Suture, site 1, day 21	4	1	2	3
Functional Suture, site 1, day 28	4	1.5	3	1.5
Functional Suture, site 2, day 7	1	4	4	1
Functional Suture, site 2, day 14	2	4	3	1
Functional Suture, site 2, day 21	1	2	4	3
Functional Suture, site 2, day 28	1.5	1.5	4	3
Area of Ulceration, day 7	2	1	4	3
Area of Ulceration, day 14	4	1	2	3
Area of Ulceration, day 21	4	1	2.5	2.5
Area of Ulceration, day 28	4	1	2	3
Area of Redness, day 7	3	1	2	4
Area of Redness, day 14	2	1	3	4
Area of Redness, day 21	1.5	1.5	3	4
Area of Redness, day 28	4	1	2.5	2.5
Tag Bulge	2	4	2	2
Mortality	4	1.5	1.5	3
AT/PITs Dropped	1.5	3	1.5	4
Percentage of Fish with Gaping, day 7	1	4	3	2
Average	2.63	2.13	2.50	2.74

4.0 Discussion

The objective of this study was to assess the performance of the bi-directional knotless tissue-closure device in relation to Monocryl™ monofilament with the simple interrupted suture pattern, which is the currently accepted technique for wound closure in juvenile salmon. SYC were implanted with JSATS ATs and PITs, and the incisions were closed using one of four suture patterns: three patterns using the knotless suture material and one suture pattern using Monocryl™ monofilament. We examined seven categorical factors including survivorship, tag loss, presence of gaping, functional suture, ulceration, redness, and tag bulging. Finally, we ranked frequency of occurrence for each factor by treatment to determine a performance index of rank (1 to 4). Generally, the elevated temperature of 17°C greatly affected all factors analyzed, thus likely indicating multiplicative stress from the surgeries and the long-term holding. The categorical factors examined significantly varied among suture pattern and treatment types and were expressed differently between the two temperature treatments. Overall, in 12°C the performance index indicated that the Second Skin performed *better* than the other treatments, although not consistently superior across the examined factors. For the 17°C group, performance index indicated that the Wide "N" overall performed *better* than the other treatments, although not consistently superior across the examined factors. For purposes of biotelemetry, in 17°C, the Second Skin treatment had the greatest gaping (days 21 and 28) and tag loss (overall), which may be of a concern when tracking juvenile Chinook in warmer water.

The bi-directional knotless suture material performance varied with the patterns examined throughout the study. In 12°C and 17°C groups, the 6-Point pattern performed consistently maintained tension across the wound more evenly than the other patterns thereby minimizing incision openness and increasing tag retention. With exception of one fish in the 12°C group, this pattern had similar incision openness on Day 0, 7, and 14 to the Second Skin, yet provided better apposition and less openness than Second Skin treatment group on days 21 and 28. However, the bi-directional knotless suture 6-Point pattern performance was at the cost of increased trauma (i.e., ulceration) as the suture moved through the tissue once the barbs become loose.

As expected with salmon, the elevated temperature treatment, 17°C, had higher mortality indicating health or stress issues. SYC have shown a thermal preference for 12.9°C (Sauter 1996), thus based on physiological tolerance polygons (Brett 1995), mortality in the 12°C (3%) group is likely related either to pre-existing condition or the stress of the surgery. Accordingly, the majority of mortalities occurred within 1 day of surgery indicating that the surgery itself was likely too stressful (e.g., anesthetic-related, surgeon generated injury). However, at 17°C, the overall experimental mortality was relatively high (48.5%) and occurred throughout the range of the observation period. Because the 17°C-related mortalities were elevated across the treatment groups (excluding the Control group), it is possible that thermal stress and concomitant surgical stress resulted in the observed short- and long-term elevated mortality rate. Likewise, Panther et al. (2011) observed no mortality in yearling, hatchery-raised Chinook salmon held at 12°C, whereas there was up to a 20% mortality rate for fish held at 20°C when testing three different incision locations. Similar to this study, Panther et al. (2011) found that the control group (no surgery) had the lowest rate of morality (~11%) in the 20°C treatment group. Therefore, it is possible that the high mortality rate is a response to thermal and surgical stress rather than a suture treatment group.

Similar to the observed rate of mortality variation between temperature groups, elevated temperature increased the rate of dropped tags. Fish held at 12°C did not experience tag loss, but one fish in the Wide “N” treatment group had an AT pushing through the incision (5.36 mm^2) by Day 28. In contrast, fish held at 17°C experienced a relatively greater (8.1%) tag loss; a total of 10 tags were dropped by Day 28. Similarly, Knights and Lasee (1996) and Bunnell and Isely (1999) also found that fish held at higher temperatures experienced significantly higher tag loss. Tissue remodeling and reduced growth have been attributed to cellular stress and higher temperature-related metabolic costs in Atlantic (*Salmo salar*) and Chinook salmon (Larsson et al. 2012; Jerrett et al. 1998), which may be partially the reason for increased damage from sutures and observed dropped tags in the 17°C exposure.

Tag retention increases with the incision margins staying approximated as the sutures remain functional (i.e., maintain proper tension and remain knotted; modified Deters et al. 2010). In theory, the 6-Point treatment should have greater tag retention because of the more uniform tension across the incision (relative to the increased number of entry and exit sites). The presence of the suture staying functional in the middle site (#2, see Figure 2.1) likely added to its effectiveness in retaining tags. When two simple interrupted sutures are used, the sutures are placed equidistant along the incision. For example, 7-mm incisions (most common length) have three sections of 2.3 mm of incision length that is anchored on the ends. This distance, 2.3 mm, is greater than the diameter of the PIT, 2.1 mm OD, allowing for the PIT tags to be dropped more easily. If the suture tension too tight or too loose and/or knots to large, the suture begins to tear at the entry exit points, it allows the gaping to increase and thus providing the opening for AT tags to drop more readily. This is likely a factor as to why the Second Skin treatment group had the highest suture retention and lowest tag retention). Conversely, the bi-directional knotless suture using the tested patterns allowed for a greater rate of tag retention likely due to the correct tension held across the incision, in particular, the middle of the incision for the first 7 days. It is possible that tag retention may be more related to proper suture tension across the incision to better approximate wound margins, instead of suture retention (i.e., sutures that remain must have correct tension) during the first few stages of wound healing. This may be even more important when salmon are exposed to elevated temperatures where there are indications of cellular stress (i.e., increase in heat shock proteins, oxidative stress response, and down regulation in genes involved with ion transport; Jefferies et al. 2012), and resultant tissue texture changes allow for greater tissue tearing and increased infection potential (Larsson et al. 2012; Jerrett et al. 1998). In addition, animals exposed to rapid pressure changes may also find the increase surface area across the incision advantageous.

The bi-directional knotless tissue-closure device outperformed the currently used Monocryl™ monofilament with the simple interrupted suture pattern with regards to incision openness. In humans, bi-directional knotless sutures decreased openness by providing a more uniformly distributed tension across the wound rather than at specific sites (Sadick et al. 1994; Shermak et al. 2009). This is consistent with our results for fish held at 12°C where, by Day 14, tissue had healed to the point where no openness was recorded. Greenburg (2010) found that the greatest degree of openness is due to unequal tension burdens being placed on the knots rather than on the length of the suture line. This tension gradient across the wound may subtly interfere with uniform healing and remodeling. Incision openness may affect mortality rates because the coelomic cavity of the fish would be exposed to the water, thereby increasing ion regulatory stress and exposure to bacteria.

Fish held at 17°C experienced more health and stress issues, which delayed healing. By Day 14, with the exception of Second Skin treatment group, the wounds in fish held at 12°C had healed and no openness was recorded. Only one fish in the Wide “N” treatment group held at 12°C had a transmitter bulging (5.36 mm^2) by Day 28. However, by Day 28 at 17°C, all treatment groups had fish with incision openness; the greatest average openness occurred in the Second Skin treatment group (range = 0 to 7.59 mm^2). Low temperatures likely delayed the appearance of tissue necrosis, macrophage response, and clearance of bacteria and necrotic muscle tissue (Anderson and Roberts 2006). These results are similar to those of Deters et al. (2010) who also found that juvenile Chinook salmon wounds healed quicker in cooler (12°C) rather than in warmer (17°C) water temperatures.

Although the Second Skin treatment had the best overall functional suture performance, all treatments had issues with suture functionality. Fish held at 12°C had greater suture retention (73.5 to 98.3%) by Day 28 at Sites 1 and 2, whereas fish held at 17°C by Day 28 had two treatments (Wide "N" and Wide "N" Knot treatments) with no remaining functional sutures at Site 2 and an overall suture retention of 0 to 77.8% at Sites 1 and 2. Walsh et al. (2000) found that by 60 days at warm temperatures (mean = 25.5°C), more than 50% of the absorbable monofilament sutures used on hybrid striped bass were expelled, whereas even by 120 days post-surgery, fish held at cold temperatures (mean = 15°C) had expelled less than 25% of sutures. Deters et al. (2010) also found suture loss was lower after 14 days in juvenile Chinook salmon held at 17°C (36%) than in fish held at 12°C (18%) when testing seven different sutures types. Similarly, Panther et al. (2010) found that suture retention in juvenile Chinook salmon is greater in lower temperatures. Bi-directional knotless sutures tend to be more rigid than traditional monofilament used in the Second Skin treatment; this likely contributes to the sutures working themselves loose on the ends and losing the desired suture pattern in the fish.

Ulceration and redness occurred in all treatment groups on all examination days. By Day 28, fish held at 12°C and 17°C the 6-Point treatment group, at both 12 and 17°C, had significantly greater ulcerated area. 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. Wagner et al. (2000) and Deters et al. (2010) found tissue trauma (number of entry/exit point) and skin-to-suture contact increases irritation. Ulceration was increased when the bi-directional knotless sutures were present but were no longer functional, creating drag and increased irritation. The "tearing" of tissue observed was related to 1) the drag created by the suture hanging out of the fish (Figure 4.1A); 2) tissue bunching resulting from the barbs moving during the swimming action of the fish (Figure 3.8, Figure 4.1B); 3) the barbs tearing the tissue immediately around the entry/exit points, eventually causing the suture to fall out of the fish (Figure 4.1C); and 4) the sutures tearing towards the incision causing the suture to fall out of the fish (Figure 4.1D).

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. As temperatures increase, suture treatment effects were diluted due to increased health or stress issues. In cooler water Second Skin is the recommended approach (2 simple sutures); while in 17°C the Wide "N" is the recommended suture. However, both the Second Skin and Wide "N" treatments had more tags dropped than with the 6-Point treatment. 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 or tissue consistency when exposed to thermal stress and suture geometry can influence retention/loss of the bi-directional knotless tissue-closure device (Ingle and King 2010; Jefferies et al. 2012). 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.

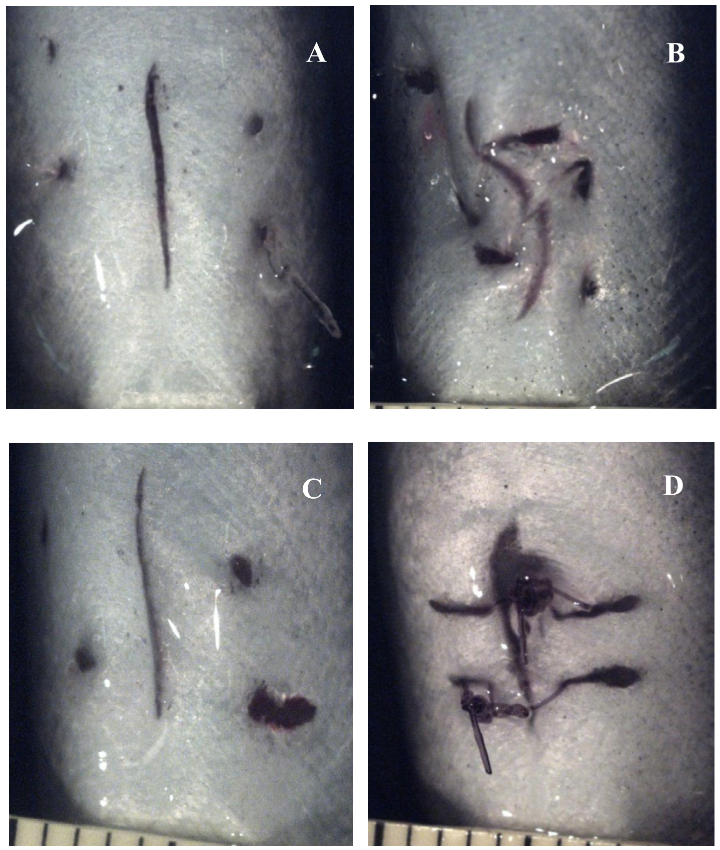

Figure 4.1. Photos Taken from Day 7 Response Examinations that Show Ulceration and Redness Often Associated with the Barbed Suture Using the Wide "N" and 6-Point Suture Patterns. A) Wide "N" pattern in SYC where the suture has slipped out of the fish creating drag. B) 6-Point suture pattern where the 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. D) Second Skin pattern where the suture has torn the tissue towards the incision.

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