Evaluation of Two Surgery Techniques for Surgical Implantation of Acoustic Transmitters in Juvenile Chinook Salmon *Oncorhynchus tshawytscha*

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Abstract – Surgically implanting a transmitter into a fish for telemetry studies can have potential effects on the growth and survival of the tagged individuals, but results are often variable based on species, size of fish, and the surgical techniques used. This study evaluated two surgery techniques for juvenile *Oncorhynchus tshawytscha* Walbaum (Chinook Salmon): the traditional sutured method and a relatively novel suture-less method where the incision is left open. Four replicate tagging trials were conducted, with each trial including 100 randomly chosen Chinook Salmon (50 control; 25 for each of the two surgery methods). Study fish were anesthetized and had a Innovasea® V5 dummy transmitter surgically implanted into the body cavity. At the completion of the 29-day trials, study fish were euthanized and assessed based on wound redness and healing at the incision site. For analyses, all the fish were pooled across the four trials ($n = 197$) and grouped by surgery type: sutured ($n = 100$) and suture-less ($n = 97$). Two-sample independent t-tests were conducted for both wound healing and redness scores between the two surgery groups. The sutured group had better scores for both wound healing ($t = -10.68$, $df = 150$, $p = <0.001$) and wound redness ($t = -7.60$, $df = 154$, $p = <0.001$) when compared to the suture-less group. The results of this study supports the use of the traditional surgical method that uses suture material to close the incision. These results contradict some recent literature that identifies the suture-less method as being more appropriate, which suggests that the effects of tag implantation may be highly variable based on surgical method used as well as the species and size of fish being studied.

Introduction

Acoustic telemetry has become a widely used tool to study fish ecology, including movement, habitat use, predator-prey interactions, post-stocking survival (e.g., Hyvarinen and Rodewald 2013; Larocque et al. 2020; Leber and Blankenship 2011), and how these behaviors are influenced by the environment (Capra et al. 2017; Cooke et al. 2004). One concern that comes with using acoustic telemetry is that this technology requires a transmitter be surgically implanted into the body cavity of a fish, which may negatively influence fish physiology and alter fish behavior (Wilson et al. 2016). Many published studies have focused on the effects of tag implantation on fish growth and survival (Brown et al. 2011; Jepsen et al. 2008; Klinard et al. 2018), but results are often variable, which suggests that a tagging evaluation prior to any telemetry study is warranted. For juvenile *Oncorhynchus tshawytscha* Walbaum (Chinook Salmon), previous research indicates that impacts of acoustic transmitter implantation may be size dependent, with more negative impacts to growth and survival noted for smaller individuals (80–109 mm Fork Length; Brown et al. 2011). In contrast, larger adult fish have shown no long-term effects on growth or survival due to acoustic transmitter implantation (Hubbard et al. 2021).

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The potential negative effects on growth and post-surgery survival are likely related to both the species and size of fish under consideration. The effects of surgery and transmitter implantation have been studied in a variety of fish families including: Percidae (Hayden et al. 2014; Weinz et al. 2020); Acerpenseridae (Auer 1999; Counihan and Frost 1999); Salmonidae (Deters et al. 2010; Thorstad et al. 2005); Centrachidae (Cooke et al. 2003); and many others (Gravenhof et al. 2020; Holbrook et al. 2012). Smaller fish are often more affected by tag implantation than larger fish due to an increased tag burden on the body (Brown et al. 2011; Hubbard et al. 2021). Effects of tag implantation likely differ among species due to differences in morphological characteristics. For example, Gravenhof et al. (2020) found surgically implanting transmitters into the ventral side of adult *Dorosoma cepedianum* Lesueur (Gizzard Shad) to be more difficult than some other species because it required prolonged surgery duration and cutting two-three rib bones to access the abdominal cavity of the fish.

How the incisions for tag implantation are closed may be another factor that influences healing and ultimately the survival of fish. The most common incision closure method uses monofilament suture material to close the wound and promote quicker healing and higher tag retention rates (Wagner and Cooke 2005). Adherence to recommended incision closure methods can minimize complications and fish discomfort and promote quicker healing (Mulcachy 2003). Some drawbacks of this recommended technique include increased inflammation, delayed wound healing, and tearing of the skin (Schoonyan et al. 2017; Wagner et al. 2000) due to its invasive nature. One alternative approach, the suture-less method, is a relatively novel technique that follows similar protocols for incision and tag insertion but does not require a suture to close the wound, rather the wound is left open. Various studies using the suture-less method have observed quicker healing rates, reduced inflammation, and improved tag retention when compared to the "traditional" sutured method (Huysman et al. 2020; Kelican et al. 2021). However, studies investigating the suture-less method are limited, with most published literature focusing on *Oncorhynchus mykiss* Walbaum (Rainbow Trout) and *Salmo trutta* Linnaeus (Brown Trout), and with adult fish rather than growing juvenile fish (Huysman et al. 2020; Kelican et al. 2021; Kientz et al. 2021).

Understanding the appropriate methods for surgical implantation of transmitters and the potential effects of implantation is crucial for ensuring that fish with an implanted transmitter both survive and behave similarly to untagged individuals when conducting a study that utilizes acoustic telemetry. Movements and habitat use of juvenile Chinook Salmon are commonly studied using acoustic telemetry (Anglea et al. 2004; Hinke et al. 2005; McMichael et al. 2006). Thus, understanding the effects and efficacy of different implantation methods can inform future studies of this species across their range. This study evaluated two surgical techniques (traditional sutured method and novel suture-less method) to inform which method may be appropriate for tagging juvenile Chinook Salmon.

Methods

Egg collection and fish rearing

The juvenile Chinook Salmon used in this study originated as eggs collected by the South Dakota Department of Game, Fish and Parks (SDGFP) at Whitlock Bay Spawning Station on Lake Oahe, South Dakota in October 2019 and 2020. After egg collection, eggs were fertilized and water hardened before being transported to Cleghorn Springs State Fish Hatchery in Rapid City, South Dakota. At the hatchery, the eggs were incubated and hatched into a flow-through system for rearing to the juvenile smolt stage. The flow-through rearing system at the hatchery consisted of 6-m diameter circular tanks with cement bottoms and stainless-steel walls with a water depth of ~0.86 m. The well water for this system has an average temperature of 11 °C, total hardness ($CaCO_3$) of 360 mg/L, a pH of 7.6, and a total dissolved solids level of 390 mg/L (South Dakota Department of Game, Fish and Parks, Ft. Pierre, SD, 2022 unpubl. data). The juvenile Chinook Salmon were fed 3-mm BioVita Fry extruded feed (Bio-Oregon, Longview, Washington, USA) at feeding rates determined using the hatchery constant method (Butterbaugh and Willoughby 1967). The juvenile salmon were reared at a density of approximately 11.36 kg/m^3.

Trial design and surgery protocols

Four replicate trials were completed for this project: two in 2020 and two in 2021. For each trial, 100 juvenile Chinook Salmon were randomly selected for inclusion in the trial (50 control; 25 for each of two surgery methods). Due to budgetary constraints, only 50 dummy transmitters were available for this study, and as such, a sample size of 50 tagged individuals was deemed sufficient for each trial. The study fish were first anesthetized using tricaine methane sulfonate (MS-222) to stage-4 anesthesia (i.e., loss of equilibrium, reflexes, and muscle tone with a slow but steady opercular rate; Summerfelt and Smith 1990). All study fish then received a unique fin clip to distinguish between control and treatment groups. All fish in the control group were then immediately returned to their rearing tanks for recovery.

The fish selected for surgery were divided into two groups: sutured and suture-less surgery methods. Fish in both groups received an incision parallel to the ventral line of the fish that was approximately 5 mm long. Incisions were made using a size #15 disposable, sterile scalpel (Cynamed®). Fish then had an Innovasea® V5 dummy transmitter (0.65 g weight in air, 12.7 mm length, and 4.3 x 5.73 mm diameter) inserted into the body cavity toward the anterior part of the fish. Fish in the suture-less group were immediately released into recovery tanks following tag insertion, while fish in the sutured group had their incision secured with one suture using a tapered point needle (RB-1) and absorbable monofilament suture material (Securos Surgical® 5-0 Securocryl; Poliglecaprone 25). The fish were held temporarily in recovery tanks until they were able to regain equilibrium, before being returned to the rearing tanks.

Surgical evaluation

For each trial, fish were monitored for 29 d (excluding trial 3, which was only conducted for 20 d due to time constraints). While unlikely, a delayed suture hypersensitivity reaction may have been missed in the trial 3 group with a shorter duration. At the completion of the trial, fish were collected from the rearing tank and euthanized using a lethal dose of MS-222. Fish were identified by treatment group based on the presence of fin clips. Digital images were captured of the incision site and used to assess wound healing and redness (Fig. 1). Wound healing and redness were assessed based on a scoring scale designed by Huysman et al. (2020) as an adaptation of a method designed by Paukert et al. (2001; Table 1). Each image was scored by two blind readers, and the mean score between readers was used for overall analyses. Fish were also dissected to determine if the dummy transmitter had been retained in the body cavity through the end of the trial. Over the course of the four trials, three study fish died due to circumstances unrelated to the study (i.e., accidentally stepping on a tagged fish within the rearing tank), and these fish were excluded from analyses.

All the surgery fish included in the study (n = 197) were pooled across the four trials and grouped by surgery type: sutured (n = 100) and suture-less (n = 97). A Jarque-Bera goodness-of-fit test was used to determine whether the sample data followed a normal distribution. Two-sample independent t-tests assuming unequal variances were conducted in

Microsoft Excel® for both the wound healing and wound redness scores, to test for potential differences between the sutured and suture-less surgery groups. Additionally, a two-sample z-test for proportions was conducted in Microsoft Excel® to test for a potential difference in tag retention proportions between the sutured and suture-less surgery groups. Statistical significance was determined at $\alpha = 0.05$

Results

The juvenile Chinook Salmon randomly selected for inclusion in the trials had a wide range in size (90–181 mm in total length (TL) and 7–87 g in weight) but mean TL (139 mm; standard error [SE] = 1 mm) and mean weight (29 g; SE = 0.7 g) were comparable across trials. This range of weights provided tag burdens (ratio of tag weight to body weight of the fish) that ranged from 0.7–9.3%, but mean tag burden was 2.6% (SE = 0.1%). Tag retention for the sutured group was significantly greater than the suture-less group across the four trials (100 v 86%; $z = 3.88$, $p < 0.001$). Results from the Jarque-Bera goodness-of-fit test indicated that the data were normally distributed for both the wound healing ($X^2 = 38.94$, df = 2, p = <0.001) and wound redness ($X^2 = 16.17$, df = 2, p = <0.001) scores. The mean wound healing score for the sutured group was 0.28 (SE = 0.04) but was 1.10 (SE = 0.07) for the suture-less group (Fig. 2); differences between the two groups were statistically significant ($t = -10.68$, df = 150, p = <0.001). Similarly, the mean wound redness score for the sutured group was 0.21 (SE = 0.04) but was 0.81 (SE = 0.07) for the suture-less treatment (Fig. 2), and both were statistically different from one another ($t = -7.60$, df = 154, p = <0.001).

Discussion

We found significantly higher tag retention rates and better wound healing and redness scores for the sutured method compared to the suture-less method. This contradicts some recent literature that has reported positive results of using a novel suture-less surgery technique (Huysman et al. 2020; Kelican et al. 2021). This difference in results could be explained by considering the life stage of study individuals. Previous published studies, such as Huysman et al. (2020) and Kelican et al. (2021), used adult fish when testing the

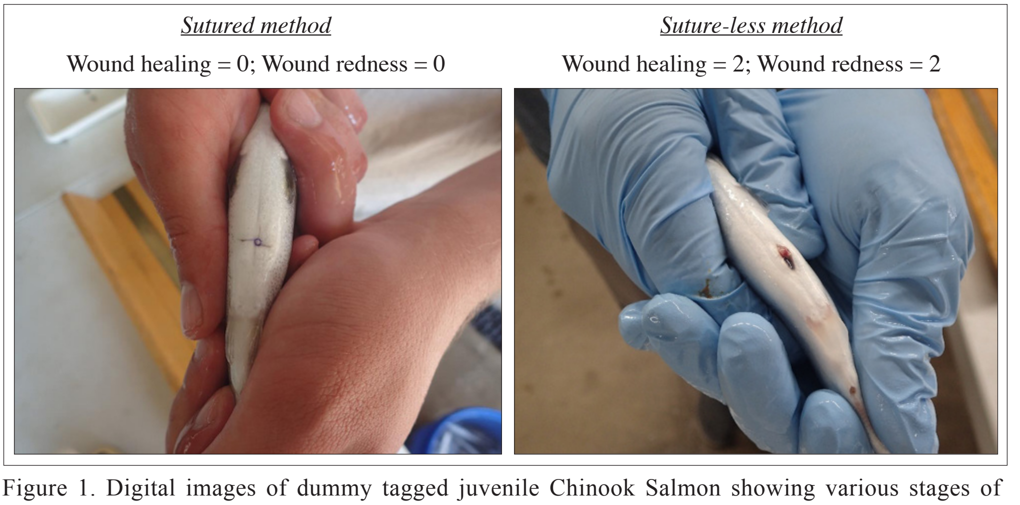

Figure 1. Digital images of dummy tagged juvenile Chinook Salmon showing various stages of healing with assigned wound healing and redness score for each using the scoring criteria outlined in Table 1. (Photos by Dylan A. Gravenhof).

Table 1. Scoring criteria developed by Huysman et al. (2020) for wound redness and wound healing used to determine the surgery method promoting quickest and best healing rates.

	Wound healing	Wound redness
0	Complete closure	No redness present
1	Closure of < 50%	Redness localized to incision/suture site
2	No closure	Redness extended beyond incision/suture site

suture-less method, but our study used smaller, juvenile individuals. This difference could suggest that the utility of the suture-less method varies based on the size and life stage of study individuals. Our results support the use of a traditional surgical procedure that uses suture material to close the incision site post-operation (Adams et al. 1998; Anglea et al. 2004; Deters et al. 2010). However, these results were somewhat surprising given the sutured method did require longer handling times and more invasive procedures. The mean surgery duration (time from when the incision was made to the time the fish was released to the recovery tank) was 69 s for the sutured method and 17 s for the suture-less method. Our results suggest that even with a handling time approximately four times longer with the traditional sutured method, the pros of a properly closed incision outweigh the potential cons of a prolonged handling time.

Some steps can be taken to mitigate any potential effects of the more invasive procedure including the use of different needle types, needle sizes, and suture material (Wagner et al. 2010). The smallest and least invasive needle should be used to easily penetrate the skin while minimizing cutting of the tissue (Von Fraunhofer and Chu 1997). Additionally, for most fish, including Chinook Salmon, absorbable monofilament is the recommended material in order to minimize tissue inflammation (Deters et al. 2010). By utilizing the appropriate surgical materials, our results suggest that the sutured method promotes better and quicker wound healing which may minimize any potential adverse effects of slightly increased handling times.

Tag retention rates are also an important factor to consider when selecting a surgery method. Acoustic transmitters are often costly, and sample sizes of tagged individuals are often smaller than comparable studies utilizing cheaper technology (Sequeira et al. 2019). Our

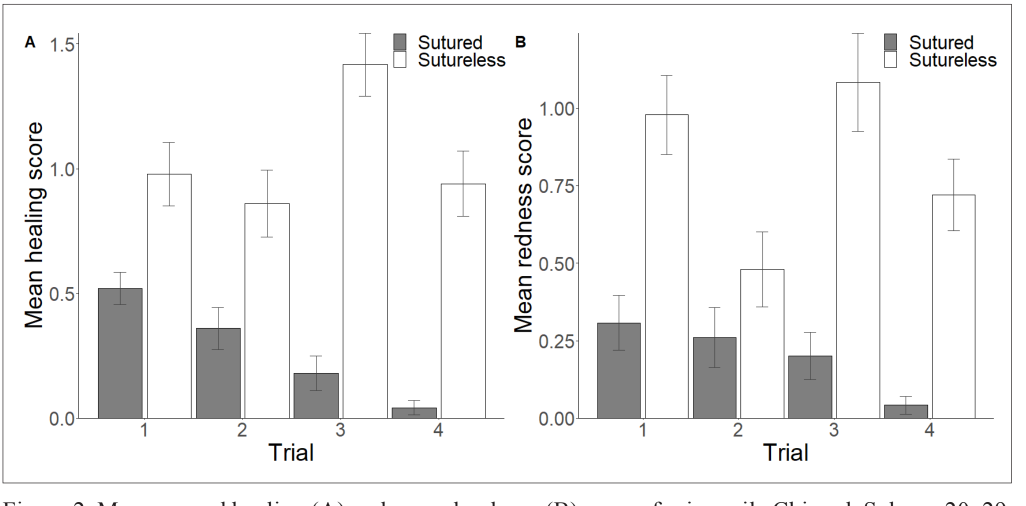

Figure 2**.** Mean wound healing (A) and wound redness (B) scores for juvenile Chinook Salmon 20–29 days post-surgery using the scoring criteria outlined in Table 1.

results did identify a significant difference in tag retentions between the two methods, which could be particularly concerning as any number of expelled transmitters has the potential to be a costly loss and negatively impact the overall results of an acoustic telemetry study.

Of the two surgery methods compared in this study, the sutured technique provided the best tag retention rates, promoted the quickest wound healing, and reduced wound redness/inflammation. One limitation of this study was relatively small sample sizes, but the results do align with what many consider the "traditional" method for surgically tagging juvenile salmonids. However, these results also contradict recent published literature that suggest suture-less surgery as a newer, better approach. These conflicting results suggest that consideration should be given to tagging protocols before starting a telemetry study. Tagging protocols may differ based on species and life stage of the fish and the type/size of the transmitter. A "one-size-fits-all" approach may not be appropriate when it comes to choosing a surgical method.

Future studies should consider replicating this study with larger sample sizes, multiple species, and both juvenile and adult fish. Our study saw conflicting results with previous studies that used different species and life stages, which suggests that more investigation could be warranted. Additionally, comparing the suture-less method in sham versus actual tag implantation surgeries could also provide insight into proper tagging techniques.

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