

Assessment of a Novel Surgical Technique for Acoustic Transmitter Insertion

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Abstract

This study compared three surgical techniques to implant acoustic transmitters in rainbow trout (*Oncorhynchus mykiss*): 1) acoustic transmitter insertion via a ventral incision closed with two sutures, 2) insertion via a ventral incision with no sutures, 3) insertion via a lateral incision with no sutures. A control group consisting of fish that were only anesthetized and handled was also included. Tag retention, wound healing, inflammation, growth, and survival were recorded over a 12-week period. Tag retention was significantly different among the treatments, at 100% in the unsutured ventral incision group, 89% in the lateral incision group, and 63% in the sutured ventral incision group. Surgical wounds in the unsutured treatment groups showed significantly less inflammation than the sutured treatment. Wound closure occurred significantly faster in the ventral incision groups compared to the lateral incision group. The results of this study show the utility of using unsutured surgical methods for implanting acoustic transmitters in rainbow trout.

Keywords

Rainbow Trout, Acoustic Transmitter, Surgery, Unsutured

1. Introduction

Electronic transmitters are frequently used to study fish ecology, behavior, and survival [1] [2]. Although transmitters can be attached externally, placing transmitters internally is preferred to reduce drag and eliminate the potential snagging of aquatic vegetation [3]. Gastro-intestinal insertion has been used, but transmitter retention is poor, and digestion can be impaired [4]. The most common method is ventral insertion into the peritoneal cavity via laparotomy, with the incision closed using sutures [5].

Professional fisheries societies recommend that surgical procedures minimize the potential introduction of microbial pathogens into the surgical wound [6]. This recommendation has typically dictated wound closure techniques, such as sutures or staples, to prevent the infiltration of water and pathogens into the wound [7]. Numerous studies have examined the use of sutures and other techniques to close the surgical wound required for transmitter insertion [5] [8] [9] [10] [11]. These techniques have been associated with inflammation, skin tearing, organ puncture, and delayed wound healing [12] [13] [14] [15].

Few studies have examined the surgical insertion of transmitters or tags into fish without the use of any type of wound closure. Prentice *et al.* [16] showed that sutures were not required to close relatively small integument breaks when inserting Passive Integrated Transponder (PIT) tags, with no tag loss in 726 fish. Similarly, Baras and Jeandrain [17] also reported 100% tag retention in European eels (*Anguilla anguilla*) with unsutured incisions. More recently, Huysman *et al.* [18] used an unsutured lateral incision to insert transmitters into rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*). They reported high tag retention with minimal or absent inflammation.

Because little research has been done on the use of sutureless incisions during transmitter insertion, there are considerable opportunities for further experimentation. This experiment builds on the positive observations using a suture-less lateral incision observed by Huysman *et al.* [18]. The objective of this study was to evaluate the use of a sutureless ventral incision, in comparison to a sutureless lateral incision, and wound closure using sutures during the insertion of acoustic transmitters into rainbow trout.

2. Material and Methods

This experiment was conducted at McNenny State Fish Hatchery (rural Spearfish, South Dakota, USA) using well water (11°C; total hardness as $CaCO_3$, 360 mg/L; alkalinity as $CaCO_3$, 210 mg/L; pH 7.6; total dissolved solids, 390 mg/L). Forty adult Shasta strain rainbow trout (mean (SE) initial length and weight = 334 (4) mm and 430 (14) g, respectively) were randomly selected from a common pool and assigned to one of four treatments: 1) Acoustic transmitter insertion via a ventral incision closed with two sutures, 2) Insertion via a ventral incision with no sutures, 3) Insertion via a lateral incision with no sutures, and 4. Control fish only anesthetized and handled.

Before surgery, each fish was anesthetized to stage IV anesthesia [19] with 60 mg/L Tricaine Methanesulfonate (MS-222; Syndel, Ferndale, Washington, USA), weighed to the nearest gram, and measured in millimeters. All surgeries were performed by one experienced surgeon [7] [20]. The fish in the ventral incision treatment groups (Treatments 1 and 2) were placed in a V-shaped trough where their gills were constantly flushed with water containing the anesthetic. After proper placement in the trough, a 10 mm incision, just large enough that the transmitter could be easily inserted, was made cranial to the pelvic groove, 3 mm

from the mid-ventral line, and just deep enough to open into the peritoneal cavity. After making the incision, a dummy acoustic transmitter (9×24 mm, 3.6 g weight in air; VEMCO, Belford Novia Scotia, Canada) was inserted through the incision and into the peritoneal cavity. Each acoustic transmitter was soaked in iodine for disinfection prior to insertion. For the sutured treatment group, two evenly spaced absorbable sutures (Oasis Nylon Monofilament sutures, 4-0, Glendora, California, USA) were placed in a simple interrupted pattern to close the surgical incision. Fish in this group were put in the recovery tank directly after the sutures were placed.

Fish in the lateral incision treatment (Treatment 3) group received a 4 mm incision approximately 10 mm cranial and in line with the pelvic fin on the left lateral side of each fish. The depth-controlled incision was made using a precision knife (X-ACTO, Elmer's Products, High Point, North Carolina, USA) disinfected in an iodine solution with only 7 mm of the blade exposed to prevent puncturing vital organs. After each incision, a dummy acoustic transmitter was inserted into the peritoneal cavity using forceps to widen the wound area. Each transmitter was disinfected in iodine prior to insertion. After transmitter insertion, fish were placed directly into the recovery tank.

Control fish did not receive any incision or transmitter but were handled and held under anesthesia for a similar amount of time as the treatment fish. After surgery and handling, fish were monitored to ensure their recovery from anesthesia and then placed back into the raceways. Mean tag to body ratios across all treatments were 0.88%, well below the recommended 2% threshold [21].

All fish in the study were placed in the same covered concrete raceway (4.7 m $long \times 2.4$ m wide and 0.5 m deep) for the duration of the experiment. One day post-surgery, the fish were fed floating feed (4.5-mm floating Oncor 80, Skretting, Tooele, Utah, USA) daily at a rate slightly above satiation. Dissolved oxygen in the raceway was maintained at or above 6.0 mg/L.

At seven-day intervals for the duration of the study, all fish were anesthetized, inspected for tag loss, measured to the nearest mm, and weighed to the nearest g. Photographs of each fish were taken to record wound healing and for individual fish identification. At the conclusion of the 12-week study, each fish was administered a lethal dose of MS-222 and a necropsy was performed to verify tag retention. The following formulas were used for analysis:

Tag retention (%)

 $= 100 \times (\text{initial number of fish tagged/final number retaining tags})$

Gain = final weight – initial weight

Specific Growth Rate(SGR)

 $= 100 \times \left[(\text{Ln end weight} - \text{Ln start weight}) / \text{number of days} \right]$

Condition Factor (K) = $10^5 \times (\text{weight/length}^3)$

All surgical site photographs were graded by four experienced fisheries pro-

fessionals. Grading was based on healing (closure) and inflammation (redness) of the wound sites using an adaptation of the Paukert *et al.* [22] method as described by Kientz *et al.* [11] (Table 1).

Data were analyzed with the SPSS (24.0) statistical analysis program (IBM, Armonk, New York, USA) using One-way Analysis of Variance. Tukey's HSD was used for post-hoc means testing. Chi-square analysis was used to analyze tag retention data. Because of the relatively small sample sizes in this study, significance was pre-determined at p < 0.10.

3. Results

At the fifth sampling week of the study, four fish were absent from the raceway, likely due to mammalian predation. None of the data from these fish were included for analysis, resulting in a reduction in the number of fish in the sutured treatment, lateral incision, and control groups to eight, nine, and nine, respectively. However, no mortalities resulting from the surgical techniques used were directly observed during the entirety of the study.

Tag retention was significantly lower in the sutured treatment in comparison to the other surgical techniques, and no tags were expelled from the trout receiving an unsutured ventral incision (**Table 2**). Necropsies performed at the conclusion of the study showed that of the 23 fish that retained tags, 10 tags were floating freely within the peritoneal cavity and 13 were encased in the soft tissue.

Final lengths, weights, and specific growth rate were not significantly different among the treatments (**Table 3**). However, the number of weeks to 50% wound closure was significantly different (**Table 4**). The unsutured lateral incision group took an average of 4.5 weeks to reach 50% closure, which was significantly different than the 2.6 weeks for the sutured ventral group, which in turn was significantly longer than the 1.6 weeks for the unsutured ventral fish. To achieve complete wound closure, the lateral incision group took a significantly longer

Table 1. Scoring criteria for wound gape severity and wound redness severity used to determing surgical wound healing for rainbow trout following dummy acoustic trasmitter insertion surgery and utilizing three different wound closure methods.

Score	Wound Healing	Wound Redness
0	Closure (complete)	No redness present
1	Closure (<50%)	Redness localized to incision/suture site
2	Closure (none)	Redness extended beyond incision/suture site

Table 2. Tag retention (%) in rainbow trout subjected to one of three surgical techniques (based on incision location). Means in a row followed by different letters are significantly different ($\chi^2 = 5.099$; p = 0.078).

	Lateral	Ventral	Sutured Ventral
Ν	9	10	8
Retention (%)	89 z	100 z	63 y

N	Control	Lateral	Ventral	Sutured Ventral	
IN -	9	9	10	8	
Initial length (mm)	333 (7)	343 (5)	339 (8)	332 (11)	
Tag/body weight (%)	N/A	0.82 (0.05)	0.85 (0.08)	0.96 (0.10)	
Intial weight (g)	410 (28)	452 (26)	456 (40)	412 (41)	
Final length (mm)	378 (10)	383 (6)	396 (8)	372 (10)	
Final weight (g)	711 (36)	745 (41)	815 (70)	690 (74)	
Weight gain (g)	322 (16)	288 (31)	359 (32)	314 (40)	
Specific growth rate	0.73 (0.04)	0.59 (0.07)	0.69 (0.02)	0.72 (0.05)	
Condition factor (K)	1.31 (0.02)	1.32 (0.04)	1.29 (0.05)	1.30 (0.05)	

Table 3. Mean (SE) length, weight, gain, speciic growth rate^a, condition factor^b, and tag-weight-to-initial-body-weight ratio for rainbow trout subjected to one of three surgical techniques (based on incision location).

a. Specific growth rate (SGR) = $100 \times [(\ln \text{ final weight} - \ln \text{ initial weight})/\text{days}];$ b. Condition factor (K) = $10^5 \times (\text{weight}/\text{length}^3)$.

Table 4. Mean (SE) weeks to wound closure and elimintaiton of inflammation in rainbow trout subjected to one of three surgical techniques (based on incision location). Means in a row followed by different letters are significantly different.

	Lateral	Ventral	Sutured Ventral	Р
Wound closure (50%)	4.5 (0.2) x	1.6 (0.2) z	2.6 (0.4) y	0.00
Wound closure (100%)	6.4 (0.1) y	4.9 (0.3) z	4.5 (0.4) z	0.00
Inflammation	0.1 (0.1) z	0.0 (0.0) z	4.8 (0.4) y	0.00

time than both ventral incision groups. Wounds across all treatment groups achieved complete closure by week seven, except in two fish which took nine and eleven weeks.

Inflammation was eliminated in the sutured ventral treatment at 4.8 weeks, which was significantly longer than the 0.1 weeks in the unsutured later treatment and 0.0 weeks in the unsutured ventral treatment. None of the fish in the unsutured ventral incision group showed visible inflammation throughout the duration of the study. **Figure 1** shows representative photographs from the three different surgical techniques from various stages of healing.

4. Discussion

The results of this study indicate the effectiveness of using ventral incisions with no sutures for acoustic transmitter insertion in rainbow trout. In addition to 100% transmitter retention, the lack of inflammation and relatively quick wound healing times make this a valid surgical technique. These results build on the observations of Huysman *et al.* [18] who reported favorable results from sutureless lateral incisions during transmitter surgeries in both rainbow trout and brown trout. In this study, the lateral incision method also showed high tag retention levels, but with significantly longer would closure times than the ventral



Figure 1. Representative photographs of the various stages of healing from rainbow trout subjected to one of three surgical techniques (based on incision location) across treatment groups. The numbers 0, 1, and 2 indicate wound healing and redness (inflammation) as described by **Table 1**.

incision. The use of lateral incisions without sutures may be beneficial when a surgical trough is not available for positioning and exposing the ventrum [7].

Relatively few articles have been reported that utilized a sutureless technique for implantation surgery. The implantation of transmitters without using sutures to close the surgical wound was first reported by Henderson *et al.* [23], who used the technique during testing of one of the first acoustic transmitters. However, their focus was just on transmitter function, not on retention or wound healing. Subsequently, Baras [24] reported improvements in wound healing using sutureless surgeries to implant tags in European barbel (*Barbusbarbus*), while Baras *et al.* [25] observed no difference in wound healing times with or without suture use during tagging surgeries in tilapia (*Oreochromis niloticus*). Conversely, Baras and Jeandrain [17] reported better tag retention and healing in yellow eels (*Anguilla anguilla*) without sutures. Lastly, Huysman *et al.* [18] reported improved healing in two salmonids using unsutured lateral incisions for transmitter insertion.

Tag retention, in conjunction with tagged-fish survival, is of the utmost importance with any surgical technique [26]. Both of the sutureless (incision-only) techniques used in this study produced excellent tag retention. Similarly, Huys-

man *et al.* [18] reported tag retentions of 100% and 84.7% in two trout species subjected to lateral incisions without sutures. The 63% tag retention observed in the sutured fish is not unusually low. In sutured rainbow trout, tag retention rates of 73% have been reported by both Bunnell and Isely [27] and Urbaniak *et al.* [28]. In addition, tag retentions in salmonids of 77.8% were observed by Kientz *et al.* [11], 85% by Welch *et al.* [29], and 88% by Bunnell and Isely [27]. While high tag-to-body ratios can negatively impact tag retention [30] [31], the ratios of less than 1.0 used in this study were all considerably less than the 2% tag-to-body ratio recommended to maximize tag retention [21] [32].

All of the wound closure times reported in this study are within the range reported previously for rainbow trout undergoing transmitter surgeries [11] [18]. However, the lateral incision did take significantly longer to heal than either of the ventral incision treatments. The reason for this difference is unknown, but sutured lateral incisions have previously been associated with increased risks of surgical complications in grass carp (*Ctenopharyngodonidella*) [33] and mortality in salmonids [34].

The lack of inflammation associated with either of the sutureless methods, combined with the presence of inflammation with the use of sutures, indicates that sutures are likely the primary cause of inflammation during surgical wound healing. Sutures are known to cause inflammation [9], likely because the fish integument responds to them as a foreign body [20]. Sutures have also consistently induced skin and muscle necrosis, causing significantly higher mortality, and slower healing [14] [17]. The absorbable monofilament used in this study is one of the least inflammatory materials [35]. It is also frequently chosen for fish surgery because it does not absorb rapidly, providing the wound time to reach complete closure [15]. However, in this study, the sutures remained beyond wound closure in every fish, likely producing prolonged inflammation [30]. By eliminating sutures and the associated inflammation, both of the sutureless surgical methods used in this study produced superior results. The inflammation results observed in this study may have been influenced by the relatively low water temperatures used. Warmer water has been associated with increased wound inflammation in salmonids and other fish species [5] [14] [27] [36].

The limitations of this study include relatively small sample sizes, conducting surgeries in a relatively controlled environment, and the use of only rainbow trout. Although the sample sizes used in this study were relatively low, the results were very consistent. Furthermore, the loss of fish likely due to mammalian predators in these relatively unsecure raceways suggests that the fish were subject to stress levels comparable to more natural environments. Lastly, although surgical techniques may differ among fish species [7], rainbow trout are considered a model organism [37]. Thus, the results of this study should at least be applicable to other salmonids, and likely have utility with other species as well.

The most accepted location for surgical procedures in fish is their ventral surface [10] [19], with most incisions occurring near the linea alba [9]. Ventral inci-

sions closed by sutures are also widely accepted among fish surgeons [38]. Thus, the use of a sutureless ventral incision for tag insertion is an improvement upon a method where fish surgeons have considerable experience. Using a sutureless technique also requires less surgical skill, expanding the range of individuals that can perform tag implantation surgeries [5]. Also, eliminating the use of sutures decreases the time of anesthesia and fish handling out-of-water, thereby decreasing fish stress and increasing recovery times [39].

Of the three surgical techniques compared in this study, the unsutured ventral incision method produced superior tag retention combined with decreased wound closure time and no inflammatory response. It fulfills the directive of Jepsen *et al.* [7] to keep fish surgical procedures as simple as possible. Given that acoustic tagging is a relatively-expensive but important part of fisheries management and is often used to make conservation and legal decisions [1], techniques to maximize tag retention and fish health are critical. The results of this study indicate that implanting tags using unsutured ventral incisions should be considered for acoustic tagging studies. Future research should focus on different fish species and sizes to further define the potential efficacy of this method of transmitter implantation.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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