

Novel Surgical Technique for Acoustic Transmitter Insertion in Rainbow Trout Reduces the Need for Surgical Training

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Abstract

The implantation of acoustic transmitters into the peritoneal cavity of fish is typically performed by experienced surgeons. This study compared the effectiveness of an experienced and inexperienced surgeon performing two different types of transmitter insertion techniques on rainbow trout (*Oncorhynchus mykiss*). The methods used were either a traditional sutured ventral incision or a novel unsutured lateral incision. There was no significant difference in surgical duration, tag retention, or fish survival between the experienced and naïve surgeon. The time to complete surgical wound closure was not significantly different between the fish operated on by an experienced or naïve surgeon, and surgeon experience had no significant effect on the duration of inflammation. However, there were significant differences between the two surgical techniques, regardless of surgeon experience. The ventral incision with sutures method took approximately three times longer to complete than the lateral incision-only method. However, complete wound closure was significantly faster in the sutured ventral incision treatment compared to the unsutured lateral treatment. Post-surgery inflammation was over seven times longer in the fish with sutures compared to those only receiving a lateral incision. Tag retention was not significantly different between the two surgical methods. The results of this study indicate that the unsutured lateral surgical technique used in this experiment can be completed with minimal training and surgical experience in contrast to the standard technique requiring sutures. These results also provide additional support to the use of sutureless surgical techniques for acoustic transmitter implantation.

Keywords

Surgery, Acoustic Transmitter, Rainbow Trout, *Oncorhynchus mykiss*

1. Introduction

Traditionally, acoustic transmitters are inserted into the peritoneal cavity via an incision at or near the ventral midline, followed by one or more sutures for wound closure [1] [2]. However, two recent studies have documented the effectiveness of using non-suture surgical techniques during the implantation of acoustic transmitters. Huysman *et al.* [3] and Kelican *et al.* [4] used a small incision on either the ventral or lateral side of the fish without any form of wound closure; sutures were not used. This incision-only technique resulted in excellent tag retention and also decreased the redness and irritation associated with the use of sutures [3] [4] [5] [6].

Huysman *et al.* [3] suggested that these unsutured techniques may reduce the need for extensive training and experience to conduct implantation surgeries. Such training, including the possible supervision of a licensed veterinarian, has been strongly recommended for those individuals conducting acoustic transmitter implantation surgeries using established methods with sutures for wound closure [2] [7] [8] [9]. Cooke *et al.* [9] suggested a rigorous three-day training schedule combined with a final assessment was necessary for untrained professionals to acquire and master the skills necessary to complete fish surgeries. Differences in post-surgery survival and suture retention have been observed between expert and novice surgeons [10]. Deters *et al.* [8] even found a marked difference in surgical outcomes from a group of four highly trained surgeons.

Because the surgical techniques described by Huysman *et al.* [3] and Kelican *et al.* [4] are relatively simple and do not require suture training, it is possible that they could be successfully used by inexperienced individuals. However, there are no published studies examining this possibility. Thus, the objective of this study was to evaluate the effect of a novice surgeon using an unsutured technique on tag retention, wound closure, and inflammation in rainbow trout receiving acoustic transmitters.

2. Materials and Methods

This experiment was conducted at McNenny State Fish Hatchery, Spearfish, South Dakota, USA using well water (11°C; total hardness as CaCO₃, 360 mg/L; alkalinity as CaCO₃, 210 mg/L; pH. 7.6; total dissolved solids, 390 mg/L). Forty adult Shasta strain rainbow trout (mean (SE) initial length 334 (4) mm and weight 430 (14) g) from a common pool were assigned to one of four treatments (n = 10): 1) sutured ventral incision by experienced surgeon; 2) lateral unsutured incision by experienced surgeon; 3) sutured ventral incision by inexperienced surgeon; and 4) lateral unsutured incision by inexperienced surgeon. All fish were held in a flow-through rectangular raceway section (4.7 m long × 2.4 m wide × 0.5 m deep) for the duration of the experiment.

2.1. Surgical Procedures

The inexperienced surgeon received approximately two hours of training on the

traditional ventral surgical technique and suturing from an experienced surgeon prior to conducting the actual surgeries for the study. During training, the experienced surgeon demonstrated the proper mechanics of acoustic transmitter surgery and post-surgery wound care, along with providing feedback while the novice practiced incisions and suturing [8]. The inexperienced surgeon only received training on the traditional ventral method. No training was given on the novel lateral technique.

Each fish was anesthetized to stage 4 anesthesia [11] with 60 mg/L Tricaine Methanesulfonate (MS-222; Syndel Ferndale, Washington, USA) and weighed and measured (total length) to the nearest g and mm. Then a unique visual implant tag (VI tag) (1.2 mm, 2.7 mm; Northwest Marine Ecology, Inc., Seattle, Washington, USA) was inserted behind the eye into the postorbital tissue for identification purposes [12]. Handling time to the nearest second was measured from when the fish were removed from the anesthetic water to when fish were placed into the raceway section after surgery.

The fish in the ventral sutured treatment were placed upside-down into a v-shaped foam trough with their gills constantly irrigated with anesthetic water. A 10-mm incision was made 3 mm from the mid-ventral line, anterior to the pelvic fins using an iodine-disinfected scalpel. Iodine disinfected inert acoustic transmitter (9 × 24 mm, 3.6 g weight in air, VEMCO, Bedford, Nova Scotia, Canada) was then inserted into the peritoneal cavity. The incisions were closed using two simple uninterrupted sutures (Oasis Monofilament absorbable sutures 4-0, Mettawa, Illinois, USA). Fish were then placed into the raceway section for recovery.

Fish in the novel lateral treatment were placed on their side on the foam pad and received a small, depth-controlled puncture/incision on their lateral side using an iodine-disinfected precision knife (X-ACTO, Elmer's Products, High Point, North Carolina, USA). The precision knife was measured off to a depth of 7 mm, deep enough to open into the peritoneal cavity but shallow enough to prevent damage to any vital organs. The incision location was made on the lateral side of the fish, approximately 1 cm anterior to the pelvic fins and in-line with the pectoral fins. Forceps were used to open the wound wide enough to insert the inert acoustic transmitter. Fish received no wound closure method and were then allowed to recover in the raceway.

2.2. Post-Surgery Rearing

Fish were fed 4.5 mm floating extruded feed daily by hand to satiation (Oncor 80, Skretting USA, Tooele, Utah, USA). Dissolved oxygen levels were above 6 mg/L throughout the course of the 12-week trial. Mean (SE) tag to body ratios were 0.91 (0.04)%, well below the recommended 2% threshold [13].

2.3. Post-Surgery Data Collection

Every seven days, the fish were anesthetized, inspected for tag loss, measured to

the nearest millimeter, and weighed to the nearest gram. The fish also had photographs taken of the surgical site for subsequent wound scoring. Each surgical site was graded by five different individuals to classify wound healing (closure) and inflammation (redness) of the wound sites using an adaptation of the Paukert *et al.* [14] method as described by Kientz *et al.* [15] (Table 1). At the end of the study, tag retention was verified by dissection.

The following formulas were used for analysis:

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

Gain = final weight – initial weight;

Specific Growth Rate (SGR) = $100 \times [(\text{Ln end weight} - \text{Ln start weight})/\text{number of days}]$;

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

2.4. Data Analysis

Data were analyzed with the SPSS (24.0) statistical analysis program (IBM, Chicago, Illinois, USA) using One-way ANOVA and Tukey's HSD post hoc means comparison test. Significance was pre-determined at $p < 0.05$. A Chi-square analysis ($p < 0.05$) was used to analyze tag retention among the treatments.

3. Results

There was no significant difference in handling (surgery) duration between the experienced and naïve surgeons (Table 2). However, there was a significant difference between surgical techniques, with the ventral incision with sutures method taking approximately three times longer to complete than the lateral incision only method. Tag retention followed a similar pattern, but there were no significant differences between the two levels of surgical experience or the two surgical methods (Table 3). Survival after surgery was not significantly different between the surgeons or technique. Only one fish died because of the surgery and tag implantation. During week three, one inexperienced ventrally-sutured fish died from surgical complications. Necropsy revealed that the surgical site had fused to the pyloric caeca. Three other fish from the experienced surgeon groups died on week five due to mammalian predation, two fish from the sutured ventral treatment and one fish from the lateral treatment.

Table 1. Scoring criteria for wound closure and wound redness severity were used to determine surgical wound healing for rainbow trout following dummy acoustic transmitter insertion surgery using two different surgical techniques with an experienced and an inexperienced surgeon.

Score	Wound Closure	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

Table 2. Mean (SE) handling time (seconds) of rainbow trout subjected to acoustic transmitter insertion surgery using two different surgical techniques with an experienced and an inexperienced surgeon. Means followed by a superscript letter are significantly different from each other ($p < 0.05$, $N = 10$).

Incision Location	Sutured	Experienced Surgeon	
		Yes	No
Ventral	Yes	202 (17) ^y	240 (18) ^y
Lateral	No	76 (9) ^z	70 (8) ^z

Table 3. Mean (SE) tag retention (%) of rainbow trout subjected to acoustic transmitter insertion surgery using two different surgical techniques with an experienced and an inexperienced surgeon ($N = 8, 9, 9$, and 10 , for the experience surgeon ventral, experience lateral, inexperience surgeon ventral, and inexperience lateral, respectively).

Incision Location	Sutured	Experienced Surgeon	
		Yes	No
Ventral	Yes	63	63
Lateral	No	89	100

At less than two weeks, wound closure to 50% was significantly quicker in the naïve surgeon sutured ventral incision group than any of the other treatments (**Table 4**). Closure to 50% was also significantly faster in both of the sutured ventral incision groups than the unsutured lateral treatments. Complete wound closure was significantly faster in ventral surgery treatments than the lateral method (**Table 5**), but there was no significant difference between the fish operated on by an experienced or naïve surgeon. Post-surgery inflammation was minimal and quickly disappeared in the lateral incision groups (**Table 6**). In contrast, the number of weeks of inflammation was over seven times longer in the fish with sutures. Surgeon experience had no significant effect on the duration of inflammation.

Visual inspection of the surgical sites for the experienced (**Image 1**) and inexperienced (**Image 2**) surgeons displayed similar healing patterns. Redness/irritation occurred along sutures, especially when sutures were retained. The incisions made by the naïve surgeon were frequently off-axis and variable in length. Sutures were frequently off-axis as well. Suture retention was 90% for the experienced surgeon compared to only 75% for the naïve surgeon. Only a single fish from the experienced surgeon lost both sutures; one suture was lost in week six and one in week nine. The naïve surgeon had three fish lose sutures. One lost both sutures in week nine, another lost one suture on week one and one suture on week seven, and the third fish lost one suture on week one but retained the other throughout the trial. The off-axis sutures and incisions did not negatively affect healing, survival, or tag retention scored between surgeons.

There was no significant effect of either surgical experience or surgical technique on ending fish length, weight, condition factor, gain, or specific growth rate.

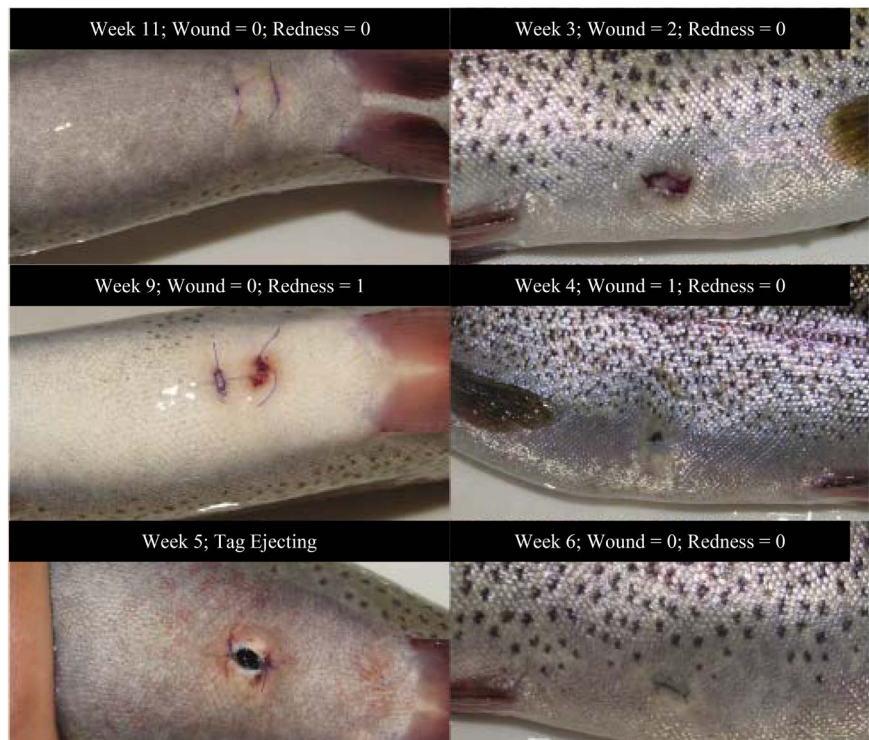


Image 1. Representative photographs of fish subjected to surgery by the experienced surgeon. The numbers on each photograph designate the wound closure and inflammation scores.

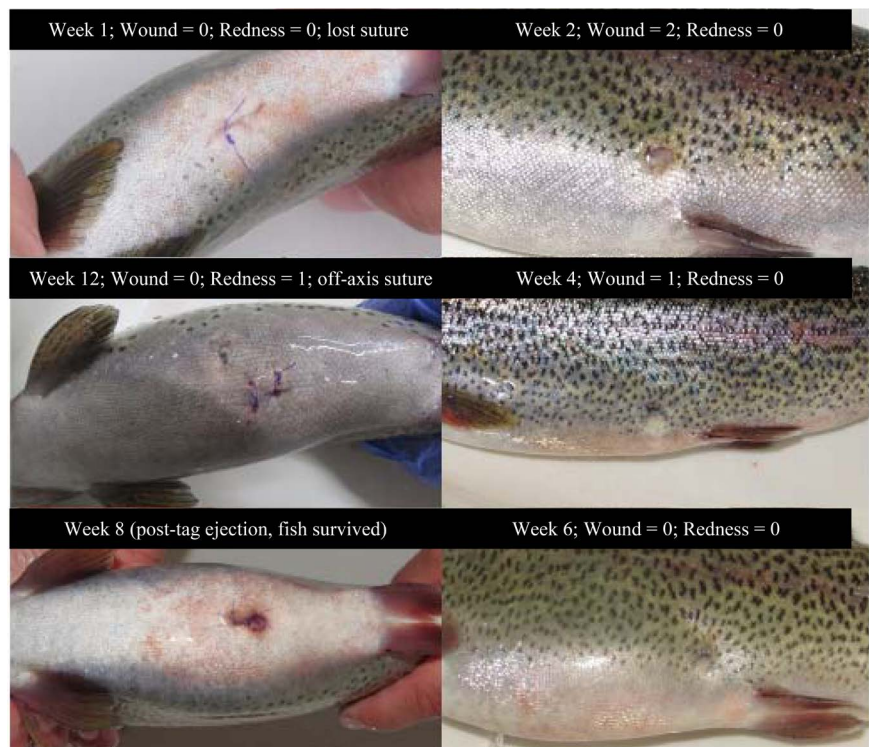


Image 2. Representative photographs of fish subjected to surgery by the inexperienced surgeon. The numbers on each photograph designate the wound closure and inflammation scores.

Table 4. Mean (SE) weeks required for 50% wound closure for rainbow trout subjected to acoustic transmitter insertion surgery using two different surgical techniques with an experienced and an inexperienced surgeon. Means followed by a superscript letter are significantly different from each other ($p < 0.05$, $N = 8, 9, 9,$ and 10 , for the experience surgeon ventral, experience lateral, inexperience surgeon ventral, and inexperience lateral, respectively).

Incision Location	Sutured	Experienced Surgeon	
		Yes	No
Ventral	Yes	2.6 (0.4) y	1.7 (0.3) z
Lateral	No	4.5 (0.2) x	3.8 (0.2) x

Table 5. Mean (SE) weeks required for complete wound closure for rainbow trout subjected to acoustic transmitter insertion surgery using two different surgical techniques with an experienced and an inexperienced surgeon. Means followed by a superscript letter are significantly different from each other ($p < 0.05$, $N = 8, 9, 9,$ and 10 , for the experience surgeon ventral, experience lateral, inexperience surgeon ventral, and inexperience lateral, respectively).

Incision Location	Sutured	Experienced Surgeon	
		Yes	No
Ventral	Yes	4.5 (0.4) z	3.9 (0.4) z
Lateral	No	6.4 (0.1) y	5.6 (0.1) y

Table 6. Mean (SE) weeks of post-surgery inflammation required for rainbow trout subjected to acoustic transmitter insertion surgery using two different surgical techniques with an experienced and an inexperienced surgeon. Means followed by a superscript letter are significantly different from each other ($p < 0.05$, $N = 8, 9, 9,$ and 10 , for the experience surgeon ventral, experience lateral, inexperience surgeon ventral, and inexperience lateral, respectively).

Incision Location	Sutured	Experienced Surgeon	
		Yes	No
Ventral	Yes	4.8 (0.4) z	5.8 (0.4) z
Lateral	No	0.1 (0.1) y	0.7 (0.2) y

4. Discussion

The results of this study support the suggestion of Huysman *et al.* [3] that the novel, non-suture, lateral surgical technique can successfully be completed by a surgeon with minimal training or experience. It took the same amount of time for both the experienced and naïve surgeon to complete the novel lateral surgery described by Huysman *et al.* [3] and Kelican *et al.* [4]. Thus, in addition to eliminating the inflammation caused by sutures, the incision-only technique is also beneficial because it eliminates the need for extensive surgical training.

With respect to traditional transmitter surgeries using ventral incisions and sutures, the results of this study also support the assertion of Deters *et al.* [8] that surgical feedback and surgeon aptitude may be essential for novice surgeons.

While the novice surgeon in this trial did not receive an extensive training protocol [9], they did receive training that included direct feedback on proper wound apposition, suture tightness, and knot making [8].

Tag retention for the ventrally sutured groups was lower than observed in other studies using salmonids. Urbaniak *et al.* [5] and Kientz *et al.* [15] reported tag retention rates for ventrally sutured rainbow trout of 73% and 78% respectively. Bunnell and Isely [6] observed rainbow trout acoustic tag retention rates ranging from 73% to 88%, depending on water temperature. Welch *et al.* [16] reported acoustic tag retention in trout of greater than 85%. In contrast, higher tag retention rates of 89% and 100% in the unsutured lateral incision groups were observed in this study. These are similar to the 85% and 100% retention rates for brown trout *Salmo trutta* and rainbow trout reported by Huysman *et al.* [3] using the same unsutured lateral incision method.

The tag-to-body ratio of 0.91% used in this study was well below the recommended 2% threshold [13] [16] [17] and should not have affected tag retention or other results. Tag retention can be negatively impacted by higher tag-to-body ratios [18] [19], although the 2% threshold has been successfully challenged many times [18] [19] [20] [21] [22].

Mulcahy [7] and Wargo Rub *et al.* [23] stated that implantation surgeries without sutures should rarely occur and must require justification. However, the results of this study, Huysman *et al.* [3], and Kelican *et al.* [4] clearly show the benefits of not using sutures during acoustic transmitter surgeries in rainbow trout at low tag-to-body ratios. In addition, better survival has been observed when Chinook salmon *Oncorhynchus tshawytscha* lost sutures [24]. Baras and Jeandrain [25] observed better tag retention in eel *Anguilla anguilla* when sutures were not used. Thus, further experimentation into the use of sutureless implantation techniques is justified.

This study was limited by using only one inexperienced surgeon. Deters *et al.* [8] found a difference among surgeons with perceivably the same amount of experience. Furthermore, this study only utilized large fish well over the 2% tag-to-body size recommendation. Further experimentation into the use of a lateral technique with other surgeons, different species of fish, and different size classes of fish is advised to determine the necessity of surgeon experience and training, as well as determine the efficacy of sutureless surgical transmitter implantation.

In conclusion, the unsutured lateral surgical technique used in this experiment can be completed with minimal training and surgical experience, in contrast to standard techniques requiring sutures. While these results may be specific to the species and size of fish used, they nonetheless provide additional support to the use of less irritating surgical techniques for acoustic transmitter insertion that do not use sutures.

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Conflicts of Interest

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

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