A comparison of implantation methods for large PIT tags or injectable acoustic transmitters in juvenile Chinook salmon

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The miniaturization of acoustic transmitters may allow greater flexibility in terms of the size and species of fish available to tag. New downsized injectable acoustic tags similar in shape to passive integrated transponder tags can be rapidly injected rather than surgically implanted through a sutured incision. Before wide-scale field use of these injectable transmitters, standard protocols to ensure the most effective and least damaging methods of implantation must be developed. Three implantation methods were tested in various sizes of juvenile Chinook salmon (Oncorhynchus tshawytscha). Methods included a needle bevel-down injection, a needle bevel-up injection with a 90° rotation, and tag implantation through an unsutured incision. Tagged fish were compared to untagged control groups. Weight and wound area were measured at tagging and every week for 3 weeks; holding tanks were checked daily for mortalities and tag losses. No significant differences among treatments were found in tag loss, or survival; but wound area was significantly reduced among fish tagged via an incision and growth was slightly reduced in bevel down fish. Although there were no significant differences, the bevel-up injection trended toward having the worst results in terms of tag loss and wound area and had high mortality. Implantaion through an incision resulted in the lowest tag loss but the highest mortality. Fish from the bevel-down treatment group had the least mortality and smaller wound areas than the bevel-up treatment group but also showed reduced growth. Cumulatively, the data suggest that the unsutured incision and bevel-down injection methods were the most effective; the drawbacks of both methods are described in detail.

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1. Introduction

In aquatic telemetry research, statistical models of behavior and survival assume that marked fish are representative of the untagged population of inference (Peven et al., 2005). However, meeting this assumption can be challenging, given that the marking process and the bearing of transmitters are known to have negative effects (Peake et al., 1997; Jepson et al., 2001; Anglea et al., 2004; Lacroix et al., 2004; Brown et al., 2006, 2010, 2013a; Welch et al., 2007; Hall et al., 2009). If marked fish are disadvantaged, study results may be biased. To minimize transmitter burden, many studies restrict the marked population to the larger individuals that meet size thresholds, especially in studies on juvenile fish (Skalski et al., 2006; Rechisky et al., 2013). However, excluding the smallest subset of a population may equally bias results (Hilborn, 2013).

Although passive integrated transponder (PIT) tags are currently one of the smallest fisheries-related tags available, acoustic transmitters are much superior in terms of detection probability; their use also allows tagged fish to be monitored in a greater diversity of locations and environments (McMichael et al., 2010; Brown et al., 2013a). Therefore, miniaturization of existing acoustic transmitters may increase flexibility in their use.

Small cylindrical acoustic transmitters (such as the juvenile salmon acoustic telemetry system [JSATS; McMichael et al., 2010]) injectable acoustic transmitter (IAT; Fig. 1B) may enable monitoring of smaller fish than previously possible and allow for less invasive and more time-efficient implantation methods than current surgical techniques. Acoustic transmitters are typically implanted through an incision that is sutured together to facilitate healing and tissue apposition and to ensure transmitter retention (Wagner et al., 2011). However, sutures can also cause tissue trauma at entry and exit points and where there is skin-to-suture contact (Wagner et al., 2011; Deters et al., 2012; Jepsen et al., 2013). This has led to research evaluating the use of fewer sutures (Boyd et al., 2007; Hall et al., 2009; Brown et al., 2010, 2013a; Welch et al., 2013).
Atlantic salmon are a species that require detailed PIT implantation methods. The literature detailing PIT implantation methods is lacking, and the best method of implanting PIT tags is rarely tested, especially for larger sizes of PIT tags. Multiple sizes of PIT tags (8–32 mm long; 1.4–3.65 mm in diameter, weighing 0.03–0.8 g) have been used for a variety of applications in fishes. Compared to surgical implantation, the shorter anesthesia times and reduced fish handling when injecting transmitters or implanting them through a non-sutured incision may lead to greater survival and a reduced tagging bias. However, before larger-scale field research is conducted using the IAT, methods of implantation should be examined across a range of fish sizes to establish a methodology for implanting PIT tags.

Especially when larger needles are used, needle orientation during initial penetration and tag injection can influence wound size (Bryson et al., 2013). Prentice et al. (1990) recommended that the bevel face away from the body of the fish during tag injection, while guidelines from the CBFWA PIT Tag Steering Committee recommend the bevel of the needle face the fish (CBFWA, 1999). Others prefer a 180° bevel rotation (bevel up to bevel down) of the needle midway through injection to verify implantation success and minimize internal damage (Gheorghiu et al., 2010). However, Bryson et al. (2013) noted that a 180° bevel rotation during insertion may impede wound healing and tag retention compared to rotating the needle only 90°.

For both injection and implantation through an incision, the size of the wound opening could influence tag retention, survival, and healing. Larger wounds may also increase the risk of pathogen invasion and impede healing (Panther et al., 2011); therefore, it is important to minimize the wound extent due to tagging. However, the most appropriate implantation method may be dependent on fish size. Tag injection is fast and efficient, but the pressure required to penetrate the body wall during the injection procedure may be important to minimize the wound extent due to tagging. However, the most appropriate implantation method may be dependent on fish size. Tag injection is fast and efficient, but the pressure required to penetrate the body wall during the injection procedure may be important to minimize the wound extent due to tagging.

The objective of this research was to compare responses of juvenile Chinook salmon along a size gradient to different methods of tagging with injectable transmitters, with the goal of minimizing
negative tagging effects. Three different implantation techniques were tested among juvenile Chinook salmon: a 3-mm unsutured incision, a bevel-down injection similar to suggestions by CBFWA (1999), and a bevel-up injection. Based on conclusions of Bryson et al. (2013), the bevel-up injection consisted of a 90° rotation following initial insertion. An unsutured incision was chosen, given the possibility for reduced handling, anesthesia time, and skin irritation.

2. Methods

2.1. Fish acquisition, holding, and tagging protocols

Juvenile fall Chinook salmon were originally obtained in November 2012 as eyed eggs from the Washington Department of Fish and Wildlife Priest Rapids Hatchery (Mattawa, WA). The eggs were hatched, and juvenile salmon were reared at the Aquatic Research Laboratory (ARL) at Pacific Northwest National Laboratory, Richland, WA. After hatching, they were held in rectangular rearing troughs (197-L raceways measuring 0.3 m × 0.3 m × 3.0 m) until they were approximately 5 months old. Then fish were held inside the ARL in 600-L circular tanks. All fish were subjected to a photoperiod of 12 h light:12 h dark and fed an ad libitum ration of Bio-Oregon (Longview, WA) pellets. Fish selected for testing were unfed for 24 h prior to tagging and prior to weekly observations.

Study fish were divided into five groups. One group of fish was unmarked and served as a true control. All other groups, including tagged groups, were individually marked with fluorescent visible implant elastomer (VIE; Northwest Marine Technology, Shaw Island, WA). Because individual growth and mortality provides a more precise metric for assessing tag and tagging effects than estimates calculated from a group of fish, we assessed individual fish in a secondary “marked” control group (similar to Brown et al., 2010). The other three treatment groups comprised the three different tag implantation methods: bevel-down injection, bevel-up injection, and incision.

For VIE marking, fish were netted from circular tanks and anesthetized with 80 mg/L of tricaine methanesulfonate (MS-222) to stage 4 anesthesia (Summerfelt and Smith, 1990), measured for fork length (FL; mm) and weight (g), and assigned to a size category, treatment, and tank based on fork length. VIE-marked fish were marked with three separate markings: in the tissue behind the right and left adipose eyelids and at the base of the dorsal fin. To account for loss of VIE tags preventing individual identification, VIE marking was performed on 10 May 2013, 5 days prior to implanting acoustic transmitters. Extra fish were tagged to replace any fish with missing VIE tags on the day of dummy transmitter implantation. After they were measured and VIE tagged, fish were placed in 10-L buckets with air stones for recovery and were then released into their respective 189-L semi-square tanks. Each of the three study tanks contained a near-equal number of fish within 3-mm size bins along a range from 66 to 108 mm to ensure a continuous range of sizes was tested (n = 72–75 per tank).

Transmitters were implanted according to the assigned method as identified by VIE color code on 15 May 2013. Both control groups (i.e., marked and unmarked) were exposed to the same protocols as tagged fish except for tagging, to ensure that all groups underwent approximately the same amount of handling. Tagged fish were intracoelomically implanted with a dummy version of the IAT. All dummy transmitters had the same proportions and weight as the real transmitters (15.0 mm long, 3.4 mm maximum diameter, 0.1 mL volume, 0.2 g in air and 0.1 g in water; Fig. 1B). Each dummy transmitter contained a PIT tag (12.5 mm long, 2.1 mm wide and 0.1 g; Destron Technologies, St. Paul, MN) to rapidly identify individual fish.

Fish were implanted with dummy transmitters according to three different methods: bevel-down injection, bevel-up injection, and incision. The two injection treatments used an 8-gauge needle (purchased from Biomark, manufactured by Vita Needle Company, Needham, MA) to inject the transmitter. Needles were 70 mm long with a 20-mm hub and a 15-mm-long vet point beveled needle point (Fig. 1A). Injections were made at the point where the tip of the pectoral fin lies against the body and 2–3 mm dorsal of the linea alba.

The bevel-down injection treatment was consistent with techniques suggested by the CBFWA PIT Tag Steering Committee (CBFWA, 1999). For this method, the bevel of the needle was open toward the abdomen of the fish. Just posterior to the point at which the tip of the pectoral fin rested against the side of the fish, the needle was inserted at a 45° angle until the skin was broken (Fig. 2A).
After the skin was broken, the needle angle was decreased to 15°, the needle was inserted until approximately one-third of the bevel was inside the fish (Fig. 2B), and the transmitter was injected.¹

The second injection treatment was a bevel-up injection with a 90° rotation (Fig. 3). This method, described in detail by Bryson et al. (2013), is commonly employed for injecting PIT tags (e.g., within the Columbia River basin; Geoffrey McMichael, Pacific Northwest National Laboratory, personal communication, May 2013). Just posterior to the point at which the tip of the pectoral fin rests against the side of the fish, the needle was inserted at a 30° angle until the skin was broken (Fig. 3A). Once the skin was broken, the needle angle was decreased to 15° and the needle was inserted until approximately half of the bevel was in the fish (Fig. 3B). The needle was then rotated 90° counterclockwise (with the needle bevel toward the linea alba), and the transmitter was injected (Fig. 3C).

For the incision implantation method, incisions were made using a BD Beaver Micro-sharp scalpel with a 15°-angled 3-mm blade (Becton, Dickinson and Company, Franklin Lakes, NJ). The incision started at the point where the tip of the pectoral fin lies against the body and was 2–3 mm above the linea alba. Cutting occurred in the posterior direction while fish were placed on a foam board covered in Fish Protector (formerly called PolyAqua; Kordon LLC, Hayward, CA; Harnish et al., 2011). The incision was measured with a ruler during cutting to ensure it was approximately 3 mm long. The tag was manually pushed through the incision into the coelom.

After tagging, fish were held in buckets of aerated water for recovery until they achieved equilibrium. They were then transferred back into the holding tanks until evaluation. Fish from the three treatment and two control groups were distributed evenly among the three tanks. All test tanks were supplied with flow-through Columbia River water at a mean temperature of 17.5°C (range 16–19°C) for the duration of the 21-day study. Dissolved oxygen levels were monitored and maintained between 98% and 103% of saturation. Mortality and tag retention were assessed and recorded daily.

Total sample size for this experiment was 208, with 41 or 42 fish in each of the five treatment groups; length, mass, and tag burdens were similar among treatments (Table 1). Fish sizes ranged from 66 to 108 mm FL (mean ± SD = 87.1 ± 12.0 mm) and weights from 2.7 to 14.6 g (mean ± SD = 7.5 ± 3.2 g). Tag burdens for the three tag treatments ranged from 1.5% to 7.3% (mean ± SD = 3.4% ± 1.6%).

2.2. Response examinations

All fish were examined immediately following transmitter implantation (day 0) and at 7, 14, and 21 days post-surgery. A single evaluator examined all fish on all examination days using a microscope. On examination days, fish were anesthetized in 80 mg/L MS-222 and then measured for FL (mm) and weight (g). Fish were then placed ventral side up on a foam-rubber pad coated with Fish Protector and supplied with a maintenance anesthesia dose of 40 mg/L MS-222. The wound extent (the area of the wound without skin coverage) was quantified for each fish. A stereo-microscope (0.65× magnification; Stemi 2000-CS, Zeiss AG, Jena, Germany) connected to a computer and monitor was used for viewing and taking images of the wound area. At the beginning of the evaluation day, a ruler at a fixed height under the microscope was calibrated with image analysis software (Image-Pro Plus and Image-Pro Analyzer, version 7.0.1, Media Cybernetics, Bethesda, MD). With the incision on the same plane as the ruler, the imaging software was used to outline the area of wound extent on examination photographs. Determination of wound area was done in real-time immediately following tagging. While one observer looked at the fish through the microscope, another researcher outlined the wound area with their guidance. The total area was then calculated in square millimeters using the Image-Pro Plus software. After the end of the study (i.e., following day 21 evaluations), all fish were euthanized with an overdose of MS-222 (250 mg/L).

2.3. Data analysis

Categorical covariates included tank and treatment; fish were held in three tanks and there were five treatments (two injection types, incision, marked controls, and unmarked controls). However, the unmarked controls were only included in analyses of overall mortality with treatment. Continuous response variables included wound area, growth, and weight at tagging and were analyzed separately for fish on days 0, 7, 14, and 21. Analysis of variance (ANOVA) was used to test for a tank effect (i.e., differences in growth and wound area by tank) as well as for differences among treatments.

¹ A video showing this technique is available on the web site of PIT tag supplier, Biomark, Inc., at http://www.biomark.com/products/videos/. Select the Tagging Tutorial option.
in tagging time, wound area (at days 0, 7, 14, and 21), and growth (change in weight from day 0 at days 7, 14, and 21). In cases where there were significant differences among tagging treatments, all pairwise differences between treatments were tested using a Bonferroni correction.

Chi-square tests examined differences in probability of tag loss and mortality among tanks and treatments. Probability of tag loss with length was analyzed using a logistic regression with a categorical response with the two possible outcomes – tag lost or tag retained. This analysis could not be performed for mortality, given the bimodality of the data (i.e., only large and small fish died).

To determine the relationships between size (weight at tagging) and response variables (wound area and growth at specific evaluation days), spline regression modeling was used. A spline point (i.e., the point at which the response variable has an abrupt shift in relationship with size) can be placed anywhere within the range of \( x \) values. The \( x \) value producing the highest \( R^2 \) value was determined to be the most accurate spline location. Because a spline regression will always find a spline point that improves the \( R^2 \) value, each spline regression was tested against a simple linear regression with an \( F \)-test to determine if the presence of a spline point improves model fit.

The analysis comparing the spline regression to a linear regression differed for wound area and growth given that for growth, unmarked controls were available for comparison. For wound area, each treatment was simply modeled using a linear regression and a spline regression (example in Fig. 4B) and the two were compared. For growth however, an \( F \)-test compared a linear regression for both treatment and control points combined to a spline regression in which treatment and control data had different intercepts but the same slope to the right (example in Fig. 4A). To the left of the spline, the control fish had a constant slope, while fish to the right of the spline and treatment fish had a different slope. For both wound area and growth, if the \( F \)-test was significant, then fitting the spline had a better model fit than not fitting the spline. Only regressions with evident spline breaks (identified in Table 2) were tested (i.e., if a spline location resulted in a dramatic decrease in growth and increase in wound area in smaller fish, as shown in the example in Fig. 4).

### 3. Results

No tank effect was observed; there were no significant \( (p < 0.3) \) differences among tanks in tag loss or mortality on any of the three evaluation days.

#### 3.1. Tag expulsion and mortality

The percentage of tag retention was highest in the incision treatment (97.0%), followed by the bevel-down (92.3%) and bevel-up treatments (86.1%). However, these differences were not significant \( (p = 0.24; \text{Fig. 5A}) \). Survival was highest in fish from the bevel-down treatment (95.1%) and lowest in fish from the incision treatment (78.5%). Survival for the bevel up treatment, marked controls and true controls were 85.7%, 90.2%, and 90.5%, respectively. However there were no significant \( (p = 0.08) \) differences in survival among the five treatments (Fig. 5C). Most mortality and tag expulsion occurred between days 12 and 21.

When tag expulsion and mortality were combined (i.e., the probability of a fish being excluded if it were being used in a field project), the bevel-down treatment had the highest percentage of fish remaining after 21 days (87.8%), followed by the incision and bevel-up treatments (76.2% and 73.6%, respectively). However, these differences were not significant \( (p = 0.24) \).

Among all tagging treatments, there was a significant \( (p < 0.001) \) effect of length on probability of tag expulsion; no fish over 77 mm FL expelled tags (Fig. 5B). Mortality, however, was more evenly distributed among size classes (Fig. 5D) and there was no significant \( (p > 0.5) \) effect of length on probability of survival.

#### 3.2. Tagging and wound area

The time required to implant the tag differed significantly \( (p < 0.001) \) among treatments. Post hoc analyses found tagging times to be similar between the two injection treatments (mean ± SD; bevel down = 19.7 ± 5.0 s, bevel up = 20.0 ± 5.4 s; \( p = 0.87 \)). However, significantly \( (p < 0.001) \) more time was needed to implant a transmitter through an incision (mean ± SD = 29.7 ± 7.3 s).

Wound areas differed significantly \( (p < 0.001) \) among treatments for all examination days (Table 3). At day 0, all pairwise comparisons were significantly different \( (p < 0.0001) \); wound areas from

### Table 2

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<th>Response</th>
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<tr>
<td></td>
<td>7</td>
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<td></td>
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</tr>
<tr>
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### Table 3

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<tr>
<th>Treatment</th>
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<tr>
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<tr>
<td>Bevel up</td>
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</tr>
<tr>
<td>Incision</td>
<td>2.0 ± 0.7</td>
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Fig. 4. Examples of spline graphs. Graphs for day 21 growth (A) and day 7 wound area (B) for incision fish against weight at tagging provide examples from combinations of specific evaluation day and treatment condition of a clear spline break (located where the vertical dashed and dotted line is present in each panel). The spline break is a result of a dramatic decrease in growth (A) or increase in wound area (B) in smaller fish. Panel A shows regression lines for day 21 growth vs. incision (solid line) and marked control fish (dashed line) plotted separately. The spline regression model from the treatment fish was tested against the linear regression model from the marked control fish. The spline regression was not significantly different from the linear regression ($p = 0.6$). In panel B, spline (solid line) and linear (dashed line) regression lines are plotted using the same data because marked controls are not available. The two regressions are significantly ($p = 0.03$) different.

Fig. 5. Tag retention in fish implanted with dummy acoustic tags using three treatments over the 21 days of the study (top left) and the number of fish losing tags within each size class during the study (top right). Survival throughout the study period (bottom left) and numbers of mortalities along a size range (bottom right) for each treatment compared to marked and unmarked (true) controls. Because true controls were not marked, the mortalities are placed in size bins associated with their size at the end of the study rather than at tagging (as with all other marked treatments).
Fig. 6. Box plots of wound areas for juvenile Chinook salmon implanted with dummy IAT tags. Wound area is shown for the day of tagging and at three consequent evaluation days. The three tagging treatments included a bevel-down injection (A), a bevel-up injection (B), and an unsutured incision (C). Box plots show median (line within box), 25th percentile (lower edge of box), 75th percentile (upper edge of box), and 1.5 × interquartile range (ends of whiskers).

bevel-up fish were the largest and those from incision fish were the smallest (Fig. 6). At day 7, results were similar to those of day 0 except that wound areas for each treatment were larger and there was no significant difference (p = 0.5) between bevel-down and incision fish (Fig. 6). However, bevel-up fish had significantly (p values < 0.001) greater wound area than either bevel-down or incision fish. At day 14, wound areas were similar (p = 0.05) between injection treatments, but incision wound areas were significantly (p < 0.001) less than both injection treatments. By day 21, all wound areas had decreased, the relative order of wound area among treatments remained the same, and all pairwise comparisons were significant (p < 0.01); incision fish had the smallest wound areas and bevel-up fish the largest.

A size-related threshold was found in the relationship between fish weight at tagging and wound extent from at least one evaluation day for all three techniques (Table 4). The spline regression identified a significant breakpoint (p values < 0.04) on three evaluation days for fish having tags implanted through an incision (days 0, 7, and 21). Spline locations (i.e., the point on the regression line at which a size threshold was observed) were fairly consistent among evaluation days, located at either 4.5 g (days 0 and 7) or 4.7 g (day 21).

A significant breakpoint was found on only one evaluation day for each of the two injection methods – with the bevel down on day 0 (p = 0.03) and with the bevel up, on day 21 (p = 0.0008). Spline locations were at 4.3 g for the bevel-down treatment and 3.9 g for the bevel-up treatment. Fork lengths and tag burdens for all spline breaks are listed in Table 4.

3.3. Growth

The only significant differences in growth among the treatments were on day 21 (p = 0.03). Seven days after tagging, marked controls had grown a mean of 0.4 g (SD = 0.5) while treatment fish had grown a mean of 0.3 g (SD = 0.3). By day 14, control fish had grown a mean of 1.5 g (SD = 1.1) compared to a mean of 1.0 (SD = 1.2) for treatment fish. By day 21, mean growth was lowest in bevel-down fish (0.7 g, SD = 1.1), followed by incision fish and marked controls (1.2, SD = 1.1 g and 1.4, SD = 1.7 g, respectively) and was highest in bevel-up fish (1.5, SD = 1.4; Fig. 7). Post hoc Bonferroni paired
comparisons at day 21 revealed no significant \( (p > 0.05) \) differences among groups, indicating that any differences among groups were marginal. However, given we detected overall significance using the ANOVA, upon applying the more liberal Fisher’s Least Significance Difference test using a non-adjusted alpha, significant differences were noted between bevel down and bevel up \( (p = 0.01) \) and between bevel down and marked control groups \( (p = 0.01) \).

There is no basis for placing a size-related threshold in the relationship between the growth of fish and their weight at tagging using any of the three tagging techniques (Table 5). On only one occasion (incision treatment on day 21) did a spline graph show the characteristic shape that may be expected, given a size threshold. However, there was no significant \( (p = 0.37) \) basis for the presence of a spline.

### 4. Discussion

Implanting dummy IATs in juvenile Chinook salmon proved least damaging when using a small incision or by injection using a bevel-down method with no rotation. The bevel-up injection with a 90° rotation had the highest levels of tag loss, largest wound area, and also had high mortality. Therefore, this discussion mainly focuses on a comparison of the unsutured incision and bevel-down injection methods and details the implications of the observed strengths and weaknesses of each.

The biggest difference between the unsutured incision and bevel-down injection was in wound area; incision fish had considerably smaller wound areas, and wound area diminished in size sooner than among bevel-down fish. Injection wounds increased in size at days 7 and 14, likely due to swelling, similar to patterns observed in Bryson et al. (2013) for injection wounds and those observed by Panther et al. (2011) for sutured incisions. Alternatively, most incision wounds began decreasing in size by day 14. Although differences among treatments in tag loss were not significant, it was expected that a larger wound area would result in higher tag loss. This pattern was apparent; fish implanted using incisions had the lowest probability of tag loss. Another notable difference among treatments was the spline regression data; only incision fish consistently showed a significant change in the relationship between wound area and fork length. These findings correspond to observations during tagging of the tag being too large in smaller fish for the incision to fully appose. That is, the presence of the tag caused the opening to round out, forming a more circular shape rather than the typically observed oblong incision shape. Consequently, there were fewer observations of full wound healing in smaller fish than larger fish. A spline pattern characteristic of a tag-effect threshold was present in bevel-down fish only on tagging day. Unlike incision wounds, there was also considerable variation in the shape of injection wound areas. For example, injection sites sometimes resulted in a small crescent-shaped wound, at other times an X shape, and sometimes eventually expanded into large circular wounds in later evaluation days. On tagging day, the significant spline regression suggests that the presence of the tag pushed the injection wound open in smaller fish. However, in later days after healing had begun, wound extent was likely more related to the initial injection wound rather than the size of the fish.

It is clear from our results that an unsutured incision can produce tag effects among smaller fish \( (<76 \text{ mm FL}, 4.5 \text{ g}, \text{ a tag burden of } 4.7\%) \). This finding is supported by significant spline regressions showing increased wound area in small fish with unsutured incisions as well as tag loss in fish less than approximately 76 mm in length. Other researchers have observed tag effects in small fish but often at tag burdens that are considerably higher than 4.7%. For example, Brown et al. (2010) found that growth and survival were negatively influenced at tag burdens of 8.2% and 6.7%, respectively, among juvenile Chinook salmon (FL = 80–109 mm) bearing a 0.74-g acoustic transmitter. Similarly, Anglea et al. (2004) observed no differences in swimming performance or predator avoidance between juvenile Chinook salmon \( (122–198 \text{ mm FL}, 22–99 \text{ g}) \) tagged with acoustic transmitters up to 6.7% of their body weight and controls. Still higher than the threshold identified in this study, other researchers determined that in salt water, maintaining tag burdens below 5.8% \( (\text{tag weight } = 1.5 \text{ g}) \) in juvenile Chinook salmon \( (110–170 \text{ mm FL}, 16.4–54.5 \text{ g}) \) reduced transmitter-related mortality (Hall et al., 2009). However, with fish as small as those tagged in this study, fish length may also be important. Lacroix et al. (2004) recommended a transmitter length of 16% of body length or less (which corresponds to an 8% tag burden for a 24-mm, 3.8-g tag) for juvenile Atlantic salmon \( (147–165 \text{ mm}, 35–45 \text{ g}) \) based on retention and swimming performance studies. Although tag burdens in our study were at a maximum 7.3% \( (66 \text{ mm FL}, 2.9 \text{ g}) \) with the 14.6-mm-long tag, the ratio of transmitter length to body length ranged from 13.5% to 22%. At the significant spline point \( (\text{i.e., } 76 \text{- mm FL}) \), the transmitter length-to-body length ratio of 19% was still higher than that recommended by Lacroix et al. (2004).

Among fish tagged using the bevel-down injection, tag loss and mortality data provide a more accurate threshold than the spline

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**Table 5**

Results from spline regression analyses that identify size-related thresholds in the relationship between growth (change in weight) after 21 days and weight at tagging of juvenile Chinook salmon implanted with dummy IAT tags using three different methods. The location of the spline (i.e., the point at which a size threshold was observed) is identified in terms of weight, fork length, and tag burden. A \( p \)-value < 0.05 indicates the spline regression is significantly different from a linear regression using growth data of control fish (individually marked with visible elastomer tags) is also shown. Box plots show median (line within box), 25th percentile (lower edge of box), 75th percentile (upper edge of box), and 1.5 \times \text{ interquartile range (ends of whiskers).}

<table>
<thead>
<tr>
<th>Spline location</th>
<th>Weight (g)</th>
<th>FL (mm)</th>
<th>TB (%)</th>
<th>( R^2 )</th>
<th>( p )</th>
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</tr>
<tr>
<td>Bevel up</td>
<td>12.5</td>
<td>104.4</td>
<td>1.7</td>
<td>0.40</td>
<td>0.43</td>
</tr>
<tr>
<td>Incision</td>
<td>4.7</td>
<td>76.9</td>
<td>4.5</td>
<td>0.37</td>
<td>0.37</td>
</tr>
</tbody>
</table>
example, Jepsen et al. (2002) suggested surgery times when two
published surgery times for acoustic tags when sutures are used. For
PIT-tagging operations (e.g., 300 fish/h [12 s per fish], according to
Brown et al. (2006)) these times are still slightly higher than those estimated for
surviving the duration of the study. Therefore, if tagging small fish is
required (<76 mm or 4.5 g, the lower limit identified for the incision
treatment), the bevel-down technique may be preferred over using an
incision.

The disparity in wound sizes among treatments was greater in
larger fish. Further, it is clear that incision wounds close much faster
than injection wounds (as evidenced from the higher occurrence of
wound areas of 0 mm² in incision fish than injection fish at day 21).
Therefore, in dynamic environments, such as when fish may pass
through hydropower structures, implanting tags through an incision
may be best, given smaller wound areas and presumably faster healing. Micro-scalpels are also easily purchased and are disposable
(i.e., do not require sharpening), but surgery times are longer. The
mean of 20 s required to tag each fish via injection is faster than inci-
sion by approximately 10 s, thereby requiring less handling time
and likely reducing the stress associated with the tagging proce-
dure. These times are still slightly higher than those estimated for
PIT-tagging operations (e.g., 300 fish/h [12 s per fish], according to
Prentice et al., 1990) but substantially lower than previously pub-
lished surgery times for acoustic tags when sutures are used. For
example, Jepsen et al. (2002) suggested surgery times when two
sutures are used should be between 1 and 2 min, and Brown et al.
(2013b) showed a reduction in surgery time from 122 s when using
two sutures to 88 s when using just one suture.

A further downside of the injection methods is related to the
needles. The surgeon in this study noted inconsistencies in design
and sharpness among newly manufactured needles that could
influence the ease of implantation and possibly the size of result-
ing wound area. These observations led to further research into the
types of factory-manufactured needles available but we noted that
many researchers do not detail exact methodology (e.g., implan-
tation location, bevel position, degree of rotation) or tools used
e.g., needle wall thickness or gauge) when injecting tags, which
is a common problem in research involving surgical procedures
in fish (Thiem et al., 2011). All needles provided to Biomark, Inc.
that were purchased for this study were produced by Vita Need-
le Company. The needles are made from tubing of a variety of
diameters and lengths, can be thick- or thin-walled, or made with
specific tip types, and are soldered into a hub that can be screwed
onto an injector. Ideally, a needle inside diameter (ID) should be
just large enough to pass the tag easily, while allowing for some
amount of error (see Vita Needle website [www.vitanneedle.com]
for availability of tubing sizes and tolerances). Regular wall thick-
ness, 8-gauge needles with a vet tip were used for this study, which
is the standard needle that Vita Needle supplies. Another possibility
moving forward may be the use of 8.5-gauge thin-walled need-
les (ID = 3.45 mm, OD = 3.96 mm, wall thickness = 0.25 mm), which
would reduce the area of the needle by 1.37 mm² and thereby posi-
ibly reduce wound size as well.

Additionally, although vet tip needles are the most commonly
used in the animal sciences (www.vitanneedle.com), there may be
better alternatives. Needle aspect and tip geometry make large dif-
fences in the insertion force required to make a cut through the
skin (Moore and Shih, 2010). When creating a needle, the primary
grind creates the bevel while the secondary grind sharpens the
needle; secondary grinds on the sides of the lumen make a vet
point, while a secondary grind on the front of the lumen is used
to make a lancet point (Fig. 8). Using the results from needles of
varying inclination and angles inserted in bovine liver, Moore et al.
(2011) developed a force model that provided insight into the dis-
tribution of tissue-cutting forces during needle insertion and found
that needles made with larger bevel angles cut with reduced force.
Therefore, use of a needle with a larger angle than the standard
vet tip (like a lancet tip) may lower insertion force and reduce
wound tearing. Conversely, the longer point created as a result of
the larger bevel angle may require deeper insertion into the coelom
to implant the tag which could increase contact area with the cut-
ing edge. We therefore recommend researchers consider exploring
available needle options if conducting a study where injectors will be
used.

Although the bevel-up method used in this study created greater
wound areas than the other techniques tested, a bevel-up inser-
tion with a rotation is the preferred method of many researchers,
given the ability to visually confirm the tag entering the fish.
Research on needle performance from the medical sciences has
suggested that needle rotation does improve needle placement
accuracy, reduces frictional forces, and creates less compression
upon insertion (Meltzner et al., 2007; Badaan et al., 2011), but
may increase tissue damage (Meltzner et al., 2007). In the med-
ical sciences, however, hollow needles are typically used to take
biopsy samples and needles are required to puncture much thicker
tissue. Han and Ehmann (2013) found that larger bevel angles
are more suited for rotational needle biopsy and therefore, a
rotation may be more appropriate if using a lancet tip injector,
which has a larger bevel than a vet tip. However, this would
require further testing. With the smaller needles and PIT tags com-
monly used for fisheries work employing the bevel-up rotation
technique, the increase in wound size due to rotation may be neg-
ligible and effects may be more pronounced with the use of larger
needles.

Despite no statistically significant differences in survival among
treatments, it was concerning to find that fish having tags
implanted through an incision had the highest number of mortal-
ities (4.5 times the number for the bevel-down injection), despite
consistently having the smallest wound openings of all treatments.
Mortality as a result of PIT-tagging typically occurs within the first
24 h (Baras et al., 1999) but the bulk of mortality observed in this
study occurred between 14 and 21 days. Only one study fish died
within 24 h (84 mm FL, 5.8 g, bevel-up treatment). The highest mor-
tality was actually in the two control groups (5% for the unmarked
control and 7% for the marked control) within the first 14 days.
The percentage of fish surviving from the incision group was equal
to that of the control groups until day 17, at which point average
wound size had decreased considerably. Even in the event of the
implantation wound being healed, it is possible that scalpels lead
to a higher probability of cutting internal organs than injectors.
It remains unknown if these mortalities were associated with the
surgical process, carrying the tags, handling, or other unidentified
factors; larger sample sizes would be needed to elucidate these
factors.

There were subtle differences in growth observed by day 21
whereby fish tagged with the bevel down method had reduced
growth. However, although the overall test was significant, post
hoc differences were only detectable when using a very liberal sta-
tistical test. This test found bevel down fish had reduced growth
compared to the bevel up treatment and unmarked controls indi-
cating that implantation method may have had a negative effect.
Differences in growth between tagged fish and controls are com-
monly observed over the short term, but tagged fish tend to show
compensatory growth and often become comparable in weight to
control fish. For example, Paukert et al. (2001) observed reduced
growth in tagged bluegill (180–200 mm FL, 2% mean tag burden)
14 days after surgery but not in subsequent weeks. Similarly,
Adams et al. (1998) observed impaired growth of juvenile Chi-
nook salmon (mean FL = 135 mm, mean tag burden = 3.6%) 21 days
after surgery but no differences 54 days after surgery. However,
Zale et al. (2005) examined small adult westslope cutthroat trout
Oncorhynhus clarkii across a range of tag burdens (0.5–5.3%) and
noted subtle decreases in growth as tag burden increased up to 6
weeks post-implantation.
The decision whether to use an incision or the bevel down injection method for IATs likely will depend on the study environment, equipment used, and surgeon training/skill. Regardless of method, tagging fish less than or equal to 3.8 g (~73 mm FL) with the 0.2-g tag we used for this research may result in high tag loss. This threshold may also increase upon assessment of other metrics or over a longer period of time. Here we measured mortality, growth, and tag retention, but these are just a few aspects of fish abilities that are usually examined to understand tag effects. Often more sensitive measures such as swimming performance, predator-avoidance abilities, fitness, or stress levels are observed (e.g., Peake et al., 1997; Jepsen et al., 2001; Anglea et al., 2004; Lacroix et al., 2004; Brown et al., 2006).

In addition, although a 3-week post-implantation examination period is suitable for typical survival studies in much of the Snake and Columbia river basins (McMichael et al., 2010), examining tagged fish over longer periods may be beneficial. For example, a longer-term assessment of wound extent could have proved informative, given that at day 21, many injection wounds still were open. It also remains to be tested if IATs will be retained across a range of sizes following exposure to challenging environments such as turbine or spillway passage at hydroelectric facilities that may expose fish to shear forces and/or pressure changes. Under these conditions, it is expected that tag expulsion and mortalities likely would be greater, and guidelines for size at tagging more conservative. For example, Boyd et al. (2011) suggested a one-suture enclosure for small acoustic transmitters, except when fish may be exposed to simulated turbine passage.

This research provides insight into appropriate tagging methods for new IATs or large PIT tags. However, we further recommend larger and longer studies to find more robust thresholds for tagging size that include more sensitive measures.

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References


