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

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ABSTRACT

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 tschawytscha*). 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. Implantation 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

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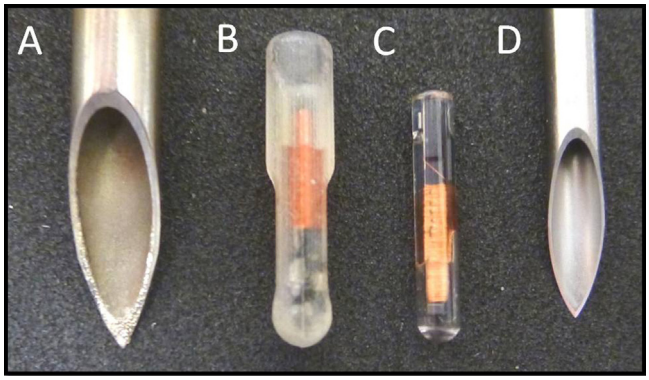

Fig. 1. A comparison of an 8-guage needle used for injecting large PIT tags (A), a dummy IAT (B), and a 12.1 mm × 2.1 mm PIT tag (C) and the 12-guage injector needle used to inject them (D).

et al., 2011; Brown et al., 2013b) as well as alternative methods such as staples (Swanberg et al., 1999) and adhesives (Petering and Johnson, 1991), for example. A transmitter similar in size and shape to PIT tags may enable researchers to move away from standard surgical procedures used for acoustic tags and adopt techniques employed for implanting PIT tags.

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), implantation methods (injection/incision), and implantation locations (intramuscular, dorsal sinus, intracoelomically) 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 implantation that is minimally invasive while ensuring transmitter retention.

As with acoustic tags, some researchers chose to implant PIT tags through an incision, and if the incision is small enough, sutures often are not used. Gries and Letcher (2002) tested the effectiveness of implanting PIT tags through unsutured incisions in age-0 Atlantic salmon *Salmo salar* and deemed it a viable alternative to using hypodermic needles, based on results of high survival (94.3%) and retention (99.8%). Incisions for PIT tag implantation are generally small, ranging from 2 to 5 mm for 12- to 32-mm PIT tags (Gries and Letcher, 2002; Zydlewski et al., 2009; Ostrand et al., 2012) and are typically made on the linea alba (Roussel et al., 2000; Gries and Letcher, 2002; Bateman et al., 2009; Chittenden et al., 2009) or parallel to the linea alba (Bateman and Gresswell, 2006). The longitudinal location of incisions is generally anterior to the pelvic fins and posterior to the pectoral fins, but exact locations vary within this area (for variations, see Roussel et al., 2000; Sigourney et al., 2005; Archdeacon et al., 2009; Dixon and Mesa, 2011).

When incision wound openings are large, it is common to use sutures. Although this requires greater handling and anesthesia times, retention of PIT tags (both 12.5 and 23 mm) when incisions are sutured has approached 100% (Baras et al., 2000; Archdeacon et al., 2009; Ficke et al., 2012). For example, Baras et al. (1999) compared the retention of PIT tags (10.3 mm × 2.1 mm, 0.07 g) in sutured and non-sutured incisions of Nile tilapia *Oreochromis niloticus* and found 100% retention among sutured fish and in the largest unsutured fish (7–15 g), whereas retention was only 86–90% in the smaller unsutured fish (2.2–7 g). However, Skov et al. (2005) compared incisions with and without sutures following implantation of a 23 mm PIT tag in small cyprinids and found those not receiving sutures had greater survival and growth rates. PIT tags are most commonly injected; Prentice et al. (1990) provide guidelines for injecting PIT tags that are widely used throughout the scientific community. For salmonids smaller than 200 mm, Prentice et al. (1990) recommend injecting PIT tags into the peritoneal cavity just posterior to the pectoral fin alongside the linea alba so that the tag lies parallel to the axis of the fish, between the pyloric caeca and pelvic girdle. In small salmonids (between 66 and 100 mm), injection using these methods has resulted in high survival and tag retention (>95%) using a 12.0 mm × 2.1 mm and 0.1-g PIT tag (Fig. 1C) and a 12-gauge (2.8-mm outside diameter [OD]) injector needle (Fig. 1D; Prentice et al., 1986, 1990). Other tagging protocols [e.g., those of the Columbia Basin Fish and Wildlife Authority (CBFWA, 1999) and Biomark, Inc., Boise, ID] have been developed using recommendations by Prentice et al. (1990), and several studies have had high survival using similar size tags, injectors, and methods (e.g., Achord et al., 1996; Ombredane et al., 1998; Dare, 2003; Acolas et al., 2007; Tatara, 2009; Gheorghiu et al., 2010). Injecting PIT tags with an outside diameter greater than 2.1 mm (e.g., Biomark model HPT22, 22 mm × 3.8 mm [Fig. 1C] or model HDX, 23 mm × 3.65 mm, Oregon RFID, Portland, OR) requires a larger needle than was used by Prentice et al. (1990) – approximately 6-gauge (Biomark N206, 5.2 mm OD) or 7-gauge (lock needle for the Oregon RFID 3.65-mm tags). The IAT (Fig. 1B) for which our study is developing guidelines requires a needle having an inside diameter (ID) of at least 3.4 mm (similar to an 8.0-gauge needle; Fig. 1A). Similar larger needles have been used with success by other researchers. For example, Bryson et al. (2013) used 8-gauge needles to inject Biomark HPT-12 STT PIT tags directly posterior to the right pectoral fin to test the feasibility of tagging juvenile Chinook salmon with large injectors and the effect of rotating the needle on wound area. Similarly, 6-gauge injectors (Biomark MK4, OD of 5.16 mm) were used to inject 23-mm-long PIT tags into the peritoneal cavity of brown trout (with a mean fish mass 90.9 ± 8.7) and yielded high survival and tag retention rates (94% and 100%, respectively; Gheorghiu et al., 2010).

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 damaging to smaller fish with more fragile tissues. For example, Baras et al. (1999) found survival of Nile tilapia injected with PIT tags to be low and proportional to fish size due to difficulties in controlling the syringe following piercing of the body wall in smaller fish.

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

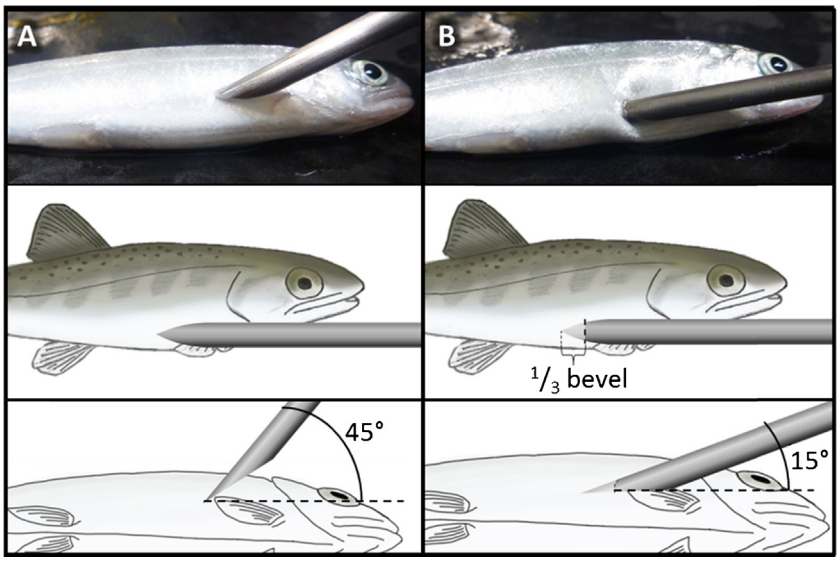

Fig. 2. A bevel-down transmitter injection. At a location just posterior to the point at which the tip of the pectoral fin rests against the side of the fish, the needle is inserted at a 45° angle until the skin is broken (A: top, middle, bottom panels). Once the skin has been broken, the needle angle is decreased to 15° and the needle is further inserted until approximately one-third of the bevel is inside the fish. Then the transmitter is injected (B: top, middle, bottom panels).

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).

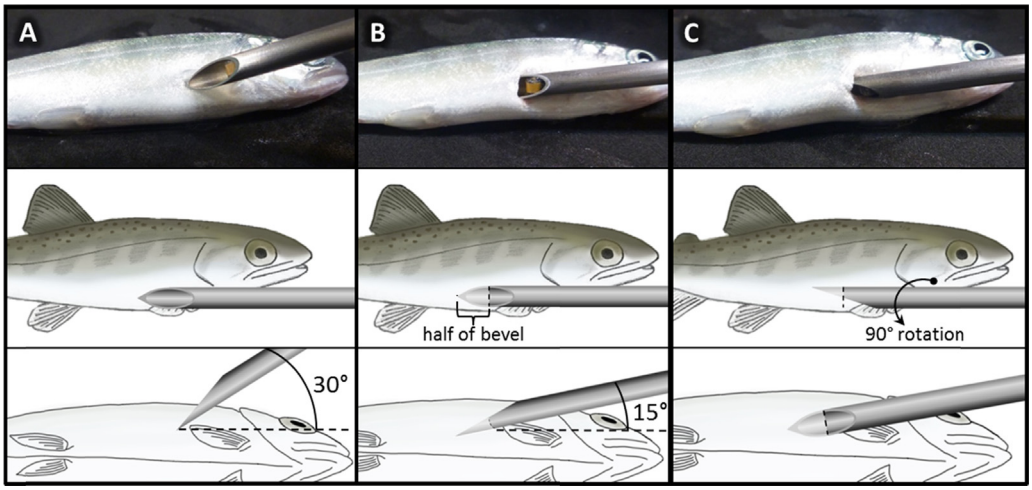

Fig. 3. A bevel-up transmitter injection. At a location just posterior to the point at which the tip of the pectoral fin rests against the side of the fish, the needle is inserted at a 30° angle until skin is broken (A: top, middle, bottom panels). Once the skin has been broken, the needle angle is decreased to a 15° angle and the needle is inserting until approximately half of the bevel is in the fish (B: top, middle, bottom panels). The needle is then rotated 90° counterclockwise (with the needle bevel toward the linea alba), and the transmitter is injected (C: top, middle bottom panels).

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 rested 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 stereomicroscope (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.

Table 1
Sizes and tag burdens for juvenile Chinook salmon within each treatment group on the day of tagging (day 0).

Treatment	*n*	Total length (mm)		Mass (g)		Tag burden (%)	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Bevel down	41	87.0 ± 11.9	66–106	7.4 ± 3.1	3.0–13.2	3.5 ± 1.5	1.6–7.1
Bevel up	42	87.2 ± 12.1	67–107	7.5 ± 3.3	2.9–14.6	3.5 ± 1.7	1.5–7.3
Incision	42	87.3 ± 12.1	67–107	7.7 ± 3.2	3.0–13.8	3.4 ± 1.6	1.5–7.1
Marked control	41	86.6 ± 12.3	66–107	7.5 ± 3.3	2.7–13.3		
Unmarked control	42	87.3 ± 12.2	66–108	7.6 ± 3.1	2.9–13.6		

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 Bonnferoni 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).

Table 2
With response variables of either wound area or growth for each of three treatments on different evaluation days, combinations for which a spline break showed a size limit threshold was apparent are identified by a √. The spline regressions of these combinations were tested against a reduced model for significance. Spline breaks are characteristic of size limit thresholds resulting from a dramatic decrease in growth and increase in wound area in smaller fish. Those combinations for which this pattern was not observed are marked by an "x" and were not tested for significance against a linear regression model.

Response	Evaluation day	Treatment		
		Bevel down	Bevel up	Incision
Wound area	0	√	×	√
	7	×	×	√
	14	×	×	√
	21	√	√	√
Growth	7	×	×	×
	14	×	×	×
	21	×	×	√

Table 3
Wound areas for juvenile Chinook salmon implanted with a dummy IAT tag according to three different methods. Measurements were taken on the day of tagging (day 0) and at three subsequent evaluation days.

Treatment	Wound area (mean ± SD, mm^2)			
	Day 0	Day 7	Day 14	Day 21
Bevel down	2.5 ± 0.4	4.7 ± 0.9	5.1 ± 1.5	1.3 ± 1.1
Bevel up	3.0 ± 0.7	6.0 ± 2.0	5.8 ± 1.3	2.5 ± 1.5
Incision	2.0 ± 0.7	4.5 ± 1.9	2.4 ± 1.8	0.4 ± 0.7

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 ($p = 0.24$; 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

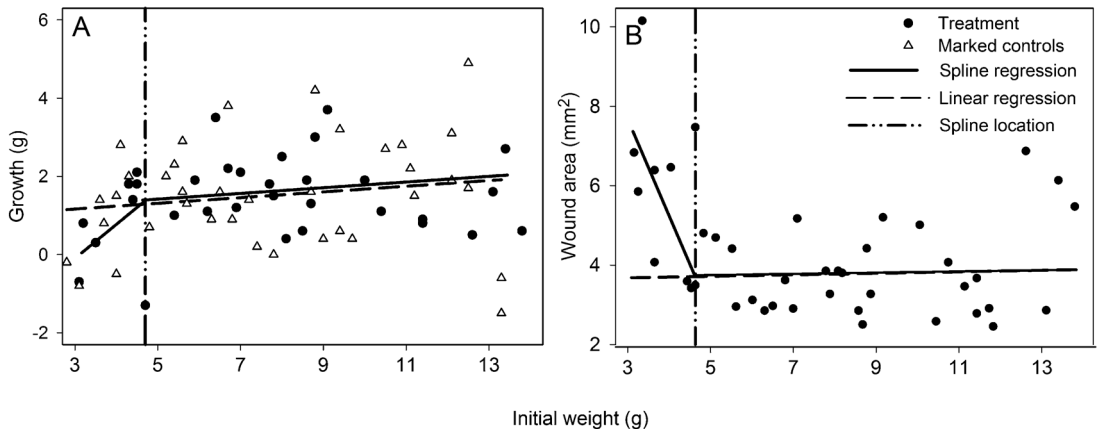

Fig. 4. Examples of spline graphs. Graphs for day 21 growth (A) and day 7 wound area (B) for incision fish against weight at tagging provide examples from combinations of specific evaluation day and treatment condition of a clear spline break (located where the vertical dashed and dotted line is present 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.

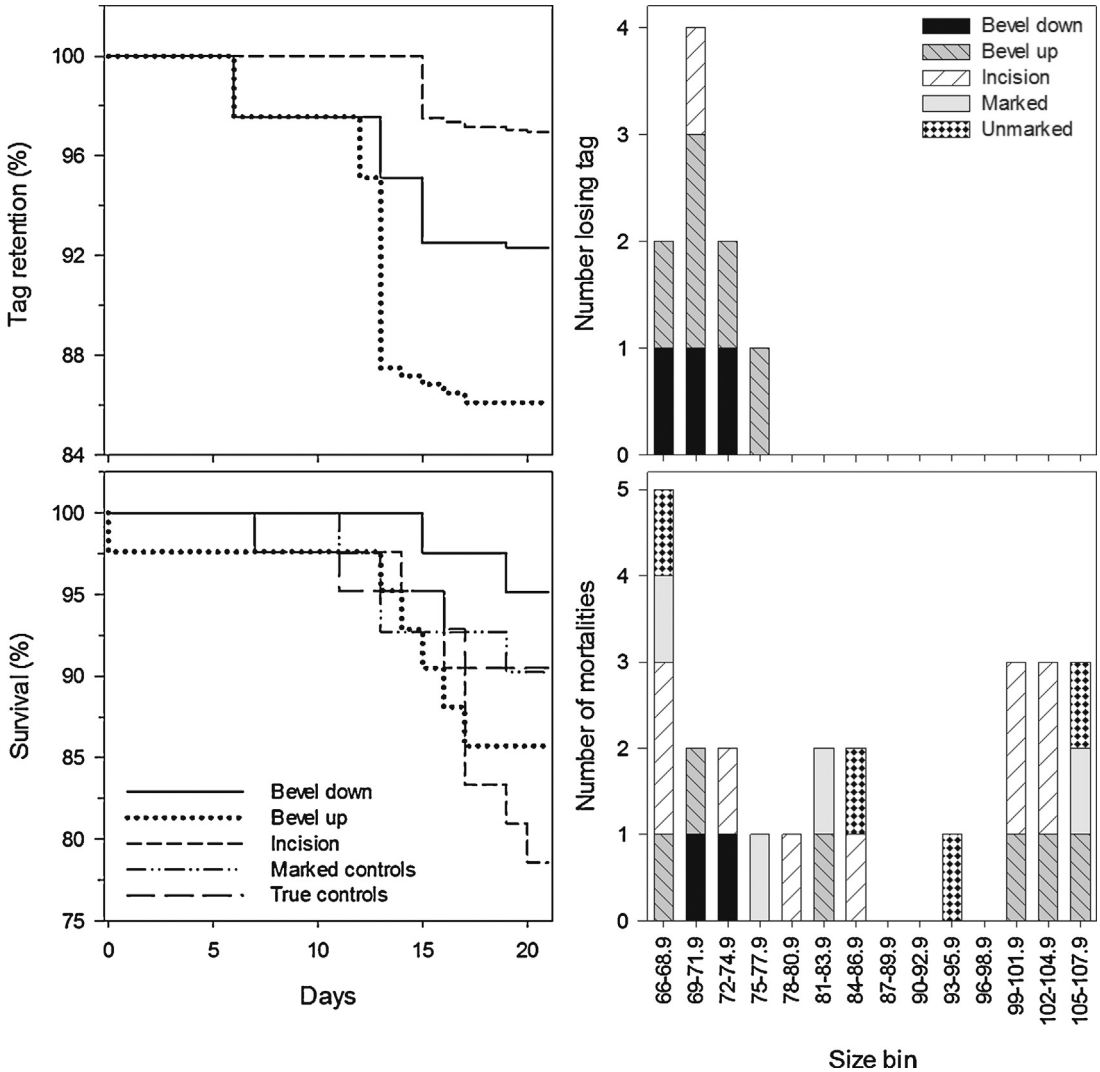

Fig. 5. Tag retention in fish implanted with dummy acoustic tags using three treatments over the 21 days of the study (top left) and the number of fish losing tags within each size class during the study (top right). Survival throughout the study period (bottom left) and numbers of mortalities along 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).

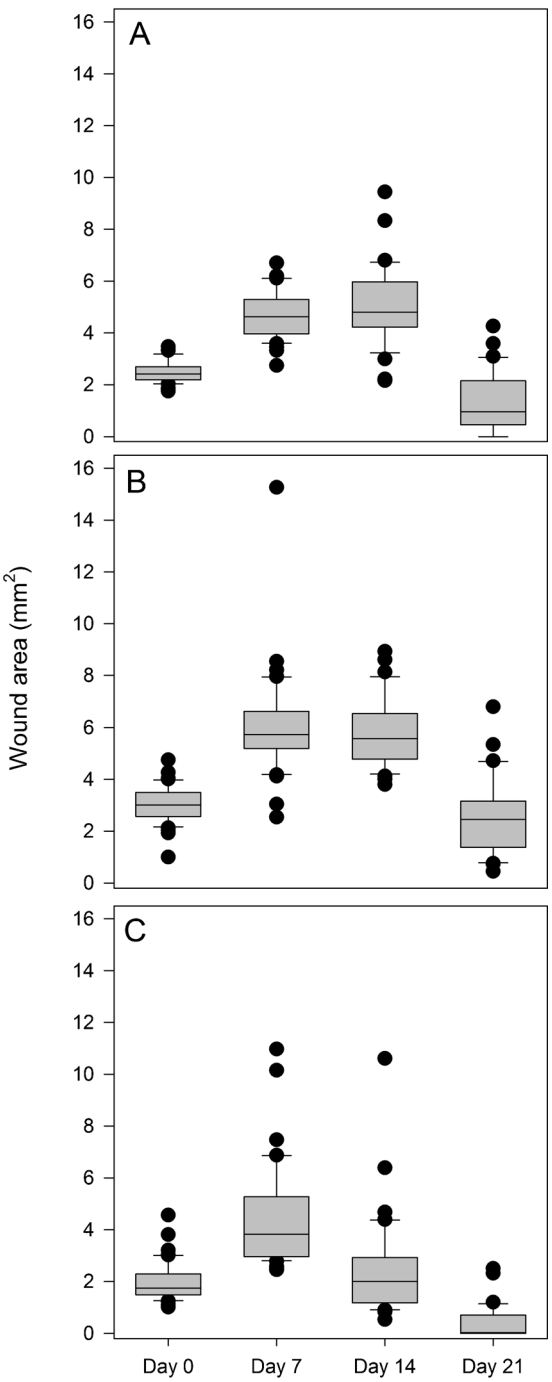

Fig. 6. Box plots of wound areas for juvenile Chinook salmon implanted with dummy IAT tags. Wound area is shown for the day of tagging and at three consequent evaluation days. The three tagging treatments included a bevel-down injection (A), a bevel-up injection (B), and an unsutured incision (C). Box 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.

Table 4
Results from spline regression analyses that identify size-related thresholds in the relationship between wound extent (at tagging and at three subsequent evaluation days) and weight at tagging for 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 a significantly better fit than a linear regression using the same data. This analysis was completed only if the spline regression showed a dramatic increase in wound area in smaller fish, characteristic of data showing a size threshold. Dashed out cells indicate that the analysis was not completed.

			Treatment		
			Bevel down	Bevel up	Incision
Day 0					
Spline location		Weight (g)	4.3	3.4	4.5
		FL (mm)	75.1	70.9	76.0
		TB (%)	5.0	6.3	4.7
	R^2		0.33	0.29	0.45
	p		0.031	–	0.002
Day 7					
Spline location		Weight (g)	7.1	3.4	4.5
		FL (mm)	86.9	70.9	76.0
		TB (%)	3.0	6.3	4.7
	R^2		0.25	0.62	0.486
	p		–	–	0.001
Day 14					
Spline location		Weight (g)	4.3	8.3	3.5
		FL (mm)	75.1	91.4	71.4
		TB (%)	5.0	2.6	6.1
	R^2		0.30	0.21	0.78
	p		–	–	0.64
Day 21					
Spline location		Weight (g)	4.4	3.9	4.7
		FL (mm)	75.6	73.3	76.9
		TB (%)	4.8	5.5	4.5
	R^2		0.29	0.48	0.72
	p		0.62	0.001	0.033

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

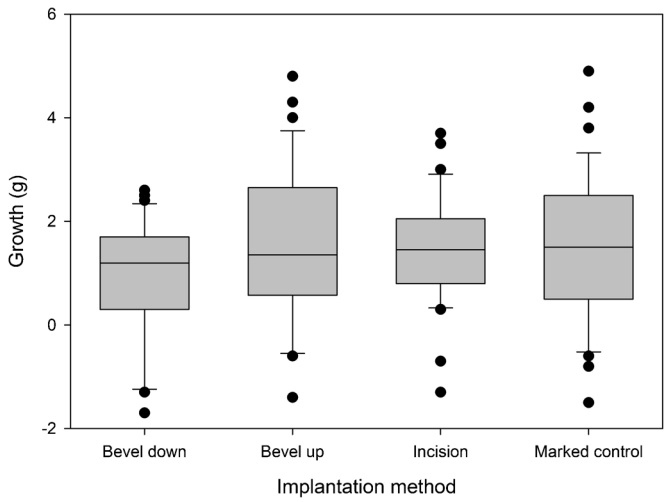

Fig. 7. Box plots of growth, calculated as the change in weight from tagging to evaluation on the last day of the study (21 days after tagging) for juvenile Chinook salmon implanted with dummy IAT tags using three different treatments (bevel-down injection, bevel-up injection, unsutured incision). Growth of marked controls (those individuals marked with visible elastomer tags but not implanted with dummy acoustic 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 × interquartile range (ends of whiskers).

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 mm FL, 4.5 g, 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 mm FL, 22–99 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% (tag weight = 1.5 g) in juvenile Chinook salmon (110–170 mm FL, 16.4–54.5 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 mm, 35–45 g) based on retention and swimming performance studies. Although tag burdens in our study were at a maximum 7.3% (66 mm FL, 2.9 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 (i.e., 76-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

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 but not implanted with transmitters). This analysis was completed only if the spline regression showed a dramatic decrease in growth in smaller fish, characteristic of data showing a size threshold.

		Treatment		
		Bevel down	Bevel up	Incision
Spline location	Weight (g)	8	12.5	4.7
	FL (mm)	90.3	104.4	76.9
	TB (%)	2.7	1.7	4.5
R^2		0.27	0.40	0.43
p		–	–	0.37

regressions. No fish over 3.8 g (72.8 mm FL; 5.6% tag burden) tagged with the bevel-down method lost tags, and all fish over 3.8 g also survived 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 mm^2 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 incision by approximately 10 s, thereby requiring less handling time and likely reducing the stress associated with the tagging procedure. 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 published 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 resulting 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., implantation 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 Needle 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.vitaneedle.com] for availability of tubing sizes and tolerances). Regular wall thickness, 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 needles (ID = 3.45 mm, OD = 3.96 mm, wall thickness = 0.25 mm), which would reduce the area of the needle by 1.37 mm^2 and thereby possibly reduce wound size as well.

Additionally, although vet tip needles are the most commonly used in the animal sciences (www.vitaneedle.com), there may be better alternatives. Needle aspect and tip geometry make large differences 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 distribution 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 cutting 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 insertion 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 (Meltsner et al., 2007; Badaan et al., 2011), but may increase tissue damage (Meltsner et al., 2007). In the medical 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 commonly used for fisheries work employing the bevel-up rotation technique, the increase in wound size due to rotation may be negligible 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 mortalities (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 mortality 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 statistical test. This test found bevel down fish had reduced growth compared to the bevel up treatment and unmarked controls indicating that implantation method may have had a negative effect. Differences in growth between tagged fish and controls are commonly 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 Chinook 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 *Oncorhynchus 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.

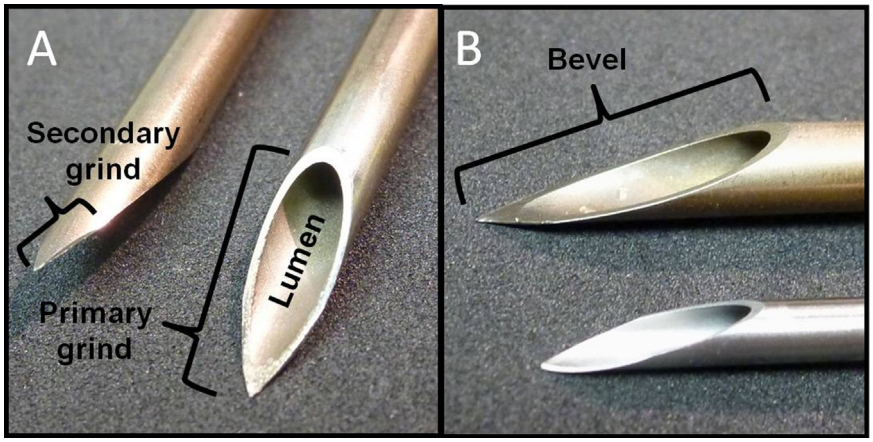

Fig. 8. Panel A illustrates parts of the needle that influence tissue cutting performance including the shape of the lumen and the locations of the primary and secondary grinds. In panel B, a side profile of an 8-guage needle (top) and a 12-gauge needle (bottom) shows the shape of the bevel being inserted into the fish.

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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