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Short communication

Comparison of 180-degree and 90-degree needle rotation to reduce wound size in PIT-injected juvenile Chinook salmon

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ABSTRACT

Most telemetry studies require the implantation or attachment of passive transponders or active transmitters to monitor and assess fish stocks and conservation to gain an understanding of fish physiology and behavior. As new telemetry technologies become available, it is imperative to study the effect of the transmitter or implantation technique on species of interest. In this study, we investigated the effects of needle axial or bevel rotation (0-, 90-, 180-degree) on wound extent and healing, and tag loss in juvenile Chinook salmon injected with an 8-gauge needle, which is required for implantation of the injectable Juvenile Salmon Acoustic Telemetry Systems acoustic transmitter and some passive integrated transponders (PITs). Although the wounds were not closed after injection (e.g., with sutures or glue), there were no mortalities, dropped tags, or indications of fungus, ulceration, or redness around the wound. No axial rotation (0-degrees) resulted in the PIT tag frequently misloading or falling out before injection. On Day 0 and post-implantation Day 7, the 90-degree bevel rotation produced a significantly smaller wound extent than the 180-degree bevel rotation. Given the wound extent compared to size of fish, we recommend researchers should consider a 90-degree rotation over the 180-degree rotation when injecting transmitters.

1. Introduction

Passive integrated transponder (PIT) technology has been used to track the movements of animals in both terrestrial and aquatic habitats. In salmonids, PIT tags have been used to estimate small- and large-scale movement in streams and are used to assess passage through hydropower facilities, such as within juvenile bypass systems, entry/exit points of estuaries, and survival through hydropower systems (Roussel et al., 2000; Gries and Letcher, 2002). Due to the wide range of applications and importance of this tracking technique, standardized protocols have been developed by BioMark (2011) and Columbia Basin Fish and Wildlife Authority (CBFWA, 1999) to ensure high-quality and consistent tagging. PIT tags are commonly injected into fish with needles ranging from 6-gauge to 12-gauge. While the injection techniques are similar, relatively few studies, compared to tag implantation requiring wound closure, have investigated intracoelomic injection techniques and resultant bio-effects in fish.

Injection studies of fish and mammals have determined needle sharpness and bevel incidence to be important factors for reducing injection bio-effects. The bevel of a needle guides the needle path as it passes through the tissue (Baumgarten, 1995), reducing the amount of force needed to penetrate the tissue, and minimizing the wound edge and ruggedness (Badaan et al., 2011). For this reason, single-use needles tend to produce smaller and cleaner incisions than multi-use needles (HHET, 2010; BioMark, 2011). Recent medical studies have indicated that needle positioning upon entry (i.e., bevel up or down) can have undesired bio-effects such as increased pain or tissue damage (Lim et al., 2012). In the Columbia River basin (USA.), the CBFWA (1999) developed a standard PIT-tagging procedure for fish using a syringe and needle, which requires the needle bevel to be down toward (or open to) the belly of the fish prior to injection so the point of the needle is away from the internal organs. The needle is not rotated on its axis during the injection; consequently the needle point is near the skin, while the bevel opening and injected PIT tag is close to the organs (Fig. 1). Many researchers use a slightly altered technique to ensure the PIT tag entered the fish (as opposed to its dropping out of the bevel or misloading in the syringe). Mueller et al. (2006) used a 180-degree axial rotation (herein referred to as "bevel rotation"; Fig. 1) to inject PIT tags into

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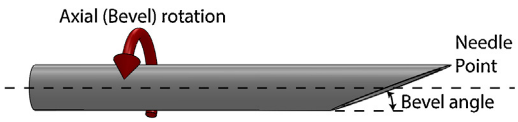

Fig. 1. Axial rotation or bevel rotation occurs as the needle is rotated in one plane in a circular motion (e.g., 90- to 180-degrees). Bevel angle is the angle formed between the prepared edge of the needle to the plane of interest, in this case the fish skin (e.g., 10- to 20-degrees).

lamprey; the needle was inserted bevel-side up until the opening was under the skin, then it was rotated bevel side down toward the lamprey body, and finally the PIT tag was injected. While injection techniques are similar, there is little indication that one technique benefits the fish more by reducing tissue damage or undesired bio-effects over time. Clearly, the latter technique, gives the operator increased confidence in technique efficacy.

As tagging techniques and systems evolve, tagging or bio-effect studies are conducted to minimize inherent differences in the susceptibility of a species to handling, anesthesia, surgical implantation of a device, and the long-term effects on their growth, behavior and survivorship. In the Columbia River basin, several studies have focused on such effects in Chinook salmon (*Oncorhynchus tshawytscha*) for PIT and Juvenile Salmon Acoustic Telemetry System (JSATS) acoustic micro-transmitters (AT) (Deters et al., 2010; Carlson et al., 2012). In 2012, a novel design of the JSATS AT was proposed as the first injectable AT. Although the design is similar to a large PIT tag, studies using this device are needed to determine both short- and long-term effects from the injection of the near-cylindrical AT. The injectable JSATS AT "proposed" outer diameter is similar to the BioMark TX1420SST PIT tags, which requires an 8-gauge needle (3.49 mm ID) that to authors' knowledge has not been used with juvenile salmon; and thus no injection procedure developed or proven to be effective.

In this study, we investigated the effects of bevel rotation on wound extent, tag loss, and wound healing in juvenile Chinook salmon injected with an 8-gauge needle required for implantation of the novel injectable AT and PIT tags. Many PIT users inject the needle with the bevel up so that the PIT tag is visible and the bevel rotation assists with the insertion of the tag without compromising tag serialization (e.g., Mueller et al., 2006), which is essential in telemetry studies. However, the action of rotating the needle with the bevel inserted 50% into the coelomic cavity, in theory, may cause more tissue damage as the bevel edge continues to cut the tissue, or if researchers inadvertently change the direction and application of force. To test this theory, we determined the optimal bevel rotation post-insertion by manipulating the mechanics of an 8-gauge hypodermic needle between 0-, 90-, and 180-degrees to deliver PIT tags into the coelomic cavity of fish; then we monitored survival, tag loss, and wound extent and healing over 14 days.

2. Material and methods

This study was conducted at the Pacific Northwest National Laboratory (PNNL), Marine Science Laboratory (MSL) in Sequim, WA using Priest Rapids Hatchery (Priest Rapids, WA) Chinook salmon. The hatchery-reared yearling Chinook salmon were held in 327-L circular holding tanks supplied with ambient flow-through aerated well water (10.2 °C) and photoperiod, and were fed at 1.3% body weight moist Bio-Diet pellets (Bio-Oregon, Longview, WA). All activities were conducted in accordance with guidelines set by the Institutional Animal Care and Use Committee, a U.S. self-regulating entity (IACUC 2011-28).

For the study, each fish was sedated in a 22.7 L bucket containing buffered tricaine methanesulfonate solution (80 mg/L MS-222; 80 mg/L $NaHCO_3$) that also contained PolyAqua (0.15 mL/L of water; Kordon Aquarium Products, Hayward, CA) until the fish did not respond to touch. While under anesthesia, fish were measured (fork length; mean 130.1 mm) and weighed (wet weight; mean 21.9 g), then randomly assigned a surgeon, treatment (control, 0-, 90-, or 180-degree bevel rotation), and a PIT tag (HPT-12 SST, BioMark, Boise, ID). To assess if prior condition or injection technique were related to mortalities, non-implanted, control fish were handled similarly to the implanted juvenile Chinook salmon.

For the treatment fish, a PIT tag was inserted into the coelomic cavity of each fish using an 8-gauge hypodermic needle and syringe loaded with PIT tag prior to injection. The surgeon inserted the needle, bevel-up, until 50% of the bevel was visible with the needle at an angle of about 10- to 20-degrees to the insertion point and fish flank, offset from the ventral midline under the posterior pectoral fin (Fig. 2a). Once the abdominal tissue was incised, the bevel was rotated based on the assigned treatment (0-, 90-, or 180-degrees) and the tag was inserted by pushing the syringe plunger (Fig. 2b and c). Two surgeons performed all tagging activities, each tagging 15 fish per treatment. Fish were photographed and placed in a recovery bucket to regain equilibrium before release into a 327-L circular tank supplied with ambient aerated well water.

Post-injection analyses occurred on Day 7 and 14, where each fish was anesthetized, scanned for PIT presence, and then the wound was photographed. Indications of infection, wound extent, and re-epithelialization were assessed on the day of injection (Day 0) and 7 and 14 days post-injection. Photos of the injection site were processed using ImageJ (Version 1.4.6, National Institutes of Health, USA, http://imagej.nih/gov/ij). Wounds were assessed by documenting the presence of re-epithelialization that provided anatomical continuity without a sustained functionality (i.e., in a stage of closing or closed, but not completely healed). Wound extent was calculated from outlining the perimeter of the wound where the scales ceased and where muscle tissue was observable. On Day 7 and 14 this measure also included the new epidermal cells covering the wound anchored to the dermis to calculate extent of regeneration (mm^2; Fig. 3).

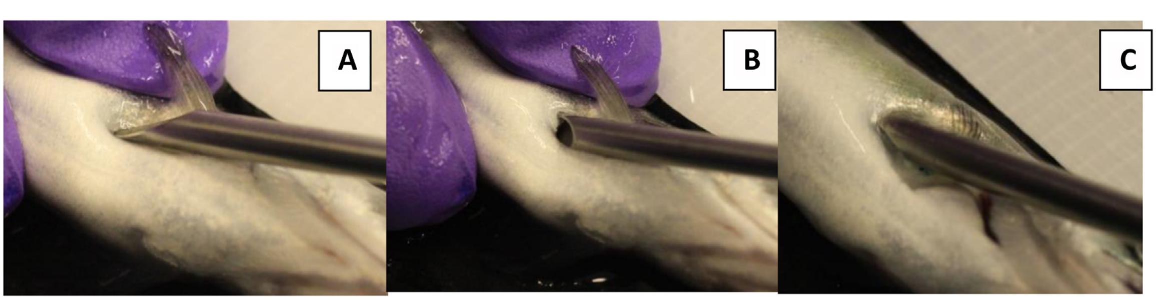

Fig. 2. Photos taken on Day 0 to demonstrate PIT implantation mechanics. Needle was inserted bevel up (A), and depending on assigned treatment, the needle was then rotated 90 degrees (B) or 180 degrees (C).

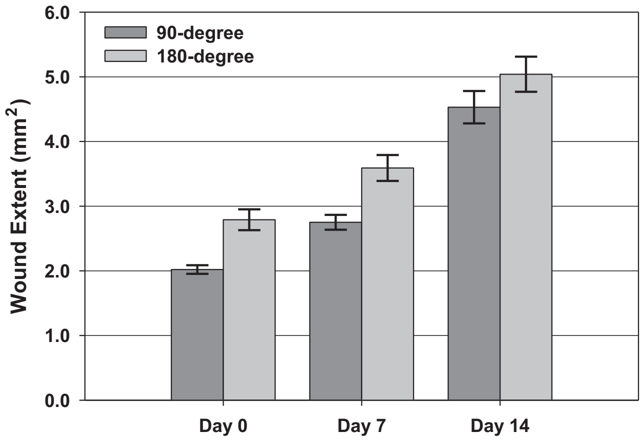

Fig. 3. Bar plot of 90-degree (light gray) and 180-degree rotation (dark gray) wound extent (mm^2) increase over experimental period. Bars on figure are standard error bars.

2.1. Statistical analysis

X^2 tests of independence were conducted to examine the difference in fish survival, external indications of infection, and tag retention rates to test for the effect of bevel rotation using an 8-gauge hypodermic needle. Repeated measures – analyses of variance (RM ANOVA) and post hoc Student's *t*-test – were used to analyze the infection and wound extent created by the bevel rotation on Day 0 and then over time (Day 0 through Day 14). Null hypotheses were rejected at $P < 0.05$.

3. Results

The lack of mortalities, external indications of ulceration or redness, and tag losses during the experiment precluded any statistical analyses on these variables. The 0-degree rotation treatment was removed due to injection complications. All fish, whether exposed to 90-degree or 180-degree bevel rotation treatments, were observed to have re-epithelialization as characterized by the wound being filled, some slightly convex, with new tissue by Day 7 or Day 14.

Mauchly's test indicated that the assumption of sphericity was not violated ($X^2(2) = 0.07$, $P < 0.05$); therefore, the degrees of freedom were not corrected. A one-way within subjects ANOVA was conducted to compare the effect of bevel rotation on wound size and thus healing over time. There was a significant effect of bevel rotation and time ($F(1,29) = 35.299$, $P < 0.001$). The Student's *t*-tests indicated 180-degree rotations had greater wound extent than 90-degree rotation on Day 0 and 7 (Day 0: Prob < t 0.018, Day 7: Prob < t 0.005), but not on Day 14 (Prob < t 0.393; Fig. 3). These results suggest that the 90-degree bevel rotation produces a smaller wound on Day 0 that also can be observed on Day 7. However, there is no difference in wound extent by Day 14, suggesting good healing of the incision.

4. Discussion

Juvenile yearling Chinook salmon implanted with a PIT using an 8-gauge hypodermic needle was a viable approach for marking fish up to 14 days. Because tags misloaded in the needle or in the incision, the tags were no longer sterile; and thus, the 0-degree bevel rotation treatment was removed from the study. Regardless of 90- or 180-degree bevel rotation, there were no mortalities, dropped tags, or indications of infection. When injecting yearling Chinook, the reduced post-insertion bevel rotation of 90-degrees resulted in smaller wound sizes for the first 7 days compared to salmon that underwent a 180-degree rotation.

Typically, mortality caused by PIT tagging occurs within the first few days post-insertion. These mortalities are often attributed to handling, internal damage from tags or needles, and/or infection from the open wounds or non-sterile tags (Baras et al., 1999; Dare, 2003). The present research limited the possibility of infection from tags and multiple-use needles by autoclaving tags prior to the study, and needles disinfected between injections with glass bead heat sterilization (10–20 s at 270–280 °C). Needles sharpness was monitored to limit tearing and excessive pressure needed to incise the fish skin. In fact, surgical PIT implantation was proposed as an alternative to avoid needle dulling effects like increased force and increase tag retention (Roussel et al., 2000; Gries and Letcher, 2002). However, our objective was to minimize wound extent to lessen bio-effects from 8-gauge needles. The use of wound closure devices (i.e., sutures) would have been contradictory to our objective as these devices increase the likelihood of external and internal infections or pulling of sutures through the skin in juvenile Chinook salmon (Deters et al., 2010).

Needle mechanics studies have indicated that bevel rotation should only occur during the initial part of the needle insertion to decrease tearing of tissue and the pull-out force required (van Gerwen et al., 2012). Generally, the practice of needle rotation in medical situations is not favored because of the increased tissue trauma it can cause, even though it assists in reducing needle friction and may increase target precision (Badaan et al., 2011; van Gerwen et al., 2012). The use of large needles to inject tags into the coelomic cavity of fish, depending on the fish size and species, takes into consideration forces that are not addressed due of the complexities of in vivo studies. For example, the axial force needed for large gauge needles to pass into the tissue, peak force to cut through tissue, and target tissue depth and consistency would require computer modeling. Even though these forces were not measured, clearly, 180-degree bevel rotation, when the needle is inserted 50% into the coelomic cavity, caused increased wound extent on Days 0 and 7 presumably from cutting the tissue as the bevel rotated compared to the 90-degree bevel rotation.

In order, to conduct this study and studies like this, assessment of tissue damage, wound size and healing were of interest; however there is no standardized method developed in fish or mammals. Moreover, the measurement of wound healing and the definition of "healed" are not well defined in the literature (Langemo et al., 2008). Wound measurements range from measuring length and width to estimate surface area to acetate tracing, photographs, and use of the Kundin wound gauge (Langemo et al., 2008). The measuring process is often complicated when measurements are conducted on live animals for which the process can create additional stress or disrupt healing (i.e., wound depth measurements), in areas difficult to measure (e.g., under pectoral fins), or when the animals require additional handling procedures (e.g., local or whole fish anesthetization). In addition, fish wound healing criteria vary from incisions healed when external layers of the body wall have "closed up" (Baras et al., 1999) to when red inflammation has subsided and/or a membrane covering the wound is observed (HHET, 2010).

Observations of each wound indicated that the incised tissue had cell regrowth and would be considered at least minimally "healed" (i.e., showing new epithelial cell proliferation over damaged muscle and viscera) by Day 7. Fish have living cells in each layer of skin allowing for quick healing (Fontenot and Neiffer, 2004), but healing is not anatomically sound. Following injury, the scales at the injury site will typically shed (as seen in the halos around the incised tissue; Fig. 2) due to the disruption of the epithelium and dermis layers. In addition, the wound edges contract and later phagocytic cells collect around the injured tissue resulting in a degenerative response to limit tissue injury and promote healing (Fontenot and Neiffer, 2004). We did not expect the wounds to fully heal (e.g., wounds not distinguishable from surrounding epithelium) within 14 days based on other suture/incision studies (Prentice et al., 1990; Panther et al., 2011). However, it demonstrates the limitation of using wound edge perimeter as an indicator of wound healing, in particular, as wound edges and new tissue growth become less discernible. A more frequent or longer-term (e.g., 21 days) resampling or improved image analysis (e.g., coupled with histology) would provide improved data resolution to determine healing rates.

The location of the insertion point and where the tags reside once they are injected have been noted to effect tag retention and growth of the tagged fish (Baras et al., 1999; Gheorghiu et al., 2010). Similar to other studies where tags were injected into the coelomic cavity, the tag retention was high, specifically 100% (Prentice et al., 1990; Dare, 2003). PIT tags often maintain their position within the coelomic cavity, frequently in the pyloric caeca, or move toward the rear and bottom of the cavity. We postulate that the long-term retention would be similar to other PIT studies where PIT tags migrate over several months away from the injection site (e.g., Gheorghiu et al., 2010). In anticipation of the injection of two tags, common in Columbia River hydropower survival studies, such as injectable PIT and AT, the insertion point was moved slightly higher so that the tags would rest lower than the incision point, and thus decrease the potential for loss of either tag. The needle bevel angle to the fish flank was kept at near 10-degrees, to minimize needle-related injuries.

In conclusion, with high tag retention rates, negligible effects on fish health and survival, an 8-gauge needle is an appropriate tool for implanting juvenile Chinook with PIT and/or injectable AT (3.4 mm OD) tags to monitor behavior, migration rates, growth, recruitment, and survival. We recommend that for intra-coelomic injection procedures, in particular those using large-gauge needles, one considers the effect of bevel rotation when the needle bevel is 50% into the coelomic cavity. When possible the reduction of the bevel rotation (i.e., from 180-degrees to 90-degrees) when inserted into the fish tissue will minimize wound extent and therefore decrease the risk of infection, tag loss, and internal damage from loss of bevel angle or needle control during rotation.

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