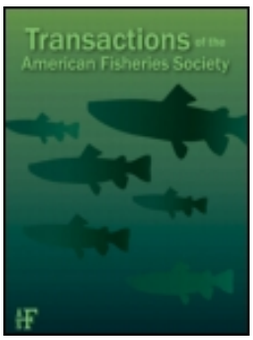

Transactions of the American Fisheries Society

ISSN: 0002-8487 (Print) 1548-8659 (Online) Journal homepage: http://www.tandfonline.com/loi/utaf20

Mortality, Transmitter Retention, Growth, and Wound Healing in Juvenile Salmon Injected with Micro Acoustic Transmitters

Stephanie A. Liss, Richard S. Brown, Katherine A. Deters, Ricardo W. Walker, Z. Daniel Deng, M. Brad Eppard, Richard L. Townsend & Adam G. Seaburg

To cite this article: Stephanie A. Liss, Richard S. Brown, Katherine A. Deters, Ricardo W. Walker, Z. Daniel Deng, M. Brad Eppard, Richard L. Townsend & Adam G. Seaburg (2016) Mortality, Transmitter Retention, Growth, and Wound Healing in Juvenile Salmon Injected with Micro Acoustic Transmitters, Transactions of the American Fisheries Society, 145:5, 1047-1058, DOI: 10.1080/00028487.2016.1176955

To link to this article: http://dx.doi.org/10.1080/00028487.2016.1176955

Published online: 16 Aug 2016.

Transactions of the American Fisheries Society 145:1047–1058, 2016

ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2016.1176955

ARTICLE

Mortality, Transmitter Retention, Growth, and Wound Healing in Juvenile Salmon Injected with Micro Acoustic Transmitters

Stephanie A. Liss,* Richard S. Brown, and Katherine A. Deters
Pacific Northwest National Laboratory, 902 Battelle Boulevard, Richland, Washington 99354, USA

Ricardo W. Walker
Pacific Northwest National Laboratory, 902 Battelle Boulevard, Richland, Washington 99354, USA; and U.S. Army Corps of Engineers, Portland District, 333 Southwest 1st Avenue #200, Portland, Oregon 97204, USA

Z. Daniel Deng
Pacific Northwest National Laboratory, 902 Battelle Boulevard, Richland, Washington 99354, USA

M. Brad Eppard
U.S. Army Corps of Engineers, Portland District, 333 Southwest 1st Avenue #200, Portland, Oregon 97204, USA

Richard L. Townsend
School of Aquatic and Fishery Sciences, University of Washington, 1325 Fourth Avenue, Suite 1820, Seattle, Washington 98101, USA

Adam G. Seaburg
School of Aquatic and Fishery Sciences, University of Washington, 1325 Fourth Avenue, Suite 1820, Seattle, Washington 98101, USA; and Genzyme Corporation, 2625 162nd Street Southwest, Lynnwood, Washington 98087, USA

Abstract

A cylindrical micro acoustic transmitter (AT; weight in air = 0.2 g) has been developed for injection into the peritoneal cavity of fish. Laboratory studies can provide tagging guidelines to minimize the effects of implantation techniques and transmitter burden (transmitter weight expressed as a proportion of fish weight) before use of the AT in field studies. To establish guidelines for minimizing tagging effects, we examined response variables (mortality, transmitter expulsion, growth, and wound healing) for micro AT-injected juvenile Chinook Salmon *Oncorhynchus tshawytscha* over a wide range of sizes (65–104 mm FL). The overarching goal was to determine a minimum size threshold at which the adverse effects of micro AT injection on fish are minimized. Juveniles (n = 700) were separated into four treatments: (1) AT injection, (2) injection of an AT and a PIT tag (AT+PIT), (3) injection of visible implant elastomer (marked control), and (4) unmarked (true) control. Fish were evaluated once per week for 4 weeks and at the end of the study (60 d posttagging). The AT- and AT+PIT-injected fish experienced greater mortality than marked controls and grew (FL and weight gain) significantly less than marked controls, although no minimum size thresholds were detected. By 60 d posttagging, the transmitter expulsion rate was 44% for AT-injected fish and 20% for AT+PIT-injected fish. However, among the 90-mm and

*Corresponding author: stephanie.liss@pnnl.gov
Received January 25, 2016; accepted April 7, 2016

larger fish in the AT treatment, none died or expelled a transmitter. Initial FL significantly affected wound healing for both injection treatments. A size threshold (85.1 mm FL) was identified for AT+PIT-injected fish at 7 d posttagging, indicating that wound areas in fish smaller than 85.1 mm were larger than the wound areas in fish exceeding 85.1 mm. Our results suggest that AT or AT+PIT injection had a greater effect on smaller juvenile Chinook Salmon than on larger fish.

As technological advances allow transmitters to become incrementally smaller, biotelemetry has become an increasingly beneficial tool for monitoring the habitat use, movement, behavior, and survival of aquatic animals in field settings (Cooke et al. 2011). Biotelemetry is particularly important for estimating the survival of migrating fishes as they pass through hydroelectric dams or other anthropogenic-associated barriers or passageways. Such studies use the survival rate of tagged individuals to make inferences about entire populations and to compare fish survival among different passage routes (Peven et al. 2005). However, the presence of transmitters or the attachment procedures used (i.e., external attachment, surgical implantation, or injection) may affect growth, swimming performance, transmitter expulsion rates, predation risk, and mortality.

Transmitter effects can also be associated with fish capture and handling or with the weight, dimensions, or volume of the transmitter. These types of transmitter effects could bias study results if they modify fish movement, behavior, or survival (Wargo Rub et al. 2014). For example, decreased swimming performance, increased risk of predation, reduced growth, transmitter expulsion, and higher levels of mortality have been associated with the surgical implantation of transmitters (Adams et al. 1998; Brown et al. 2006; Jepsen et al. 2008a; Deters et al. 2010; Walker et al. 2016). To reduce potential transmitter effects, researchers conducting telemetry studies may implant transmitters into only the larger individuals of a population (thus reducing transmitter burden [transmitter weight expressed as a proportion of fish weight]), which could lead to biased results (Skalski et al. 2006; Rechisky et al. 2013). For example, previous studies have required minimum length criteria (e.g., ≥95 mm FL) for the implantation of acoustic transmitters into Chinook Salmon *Oncorhynchus tshawytscha* (McMichael et al. 2011; SPSC 2011). In addition, there are guidelines for implanting PIT tags into fish: juvenile salmonids should be at least 65 mm FL, and the optimal tagging size is 80–150 mm FL (PTSC 2014). This finding is similar to that of Larsen et al. (2013), who found an optimal tagging size of 80 mm FL and larger for Atlantic Salmon *Salmo salar*. As such, a reduction in transmitter size (and therefore transmitter burden) could make it possible to implant transmitters into smaller individuals, thereby encompassing a greater proportion of the population while minimizing transmitter effects.

Recently, a novel cylindrical micro acoustic transmitter (AT) was developed (diameter = 3.4 mm; length = 15 mm; weight in air = 0.2 g; weight in water = 0.1 g; volume = 0.1 mL; battery life > 100 d at a 3-s ping rate; Chen et al. 2014; Deng et al. 2015). Smaller transmitter size makes it possible to implant the ATs into smaller fish, which can better represent the wild run-of-the-river fish (e.g., out-migrating Chinook Salmon in the Columbia River basin as small as ~55 mm FL). However, laboratory-based survival and growth studies are critical for understanding how fish of various sizes are able to recover from transmitter injection and for determining their rates of transmitter retention (Jepsen et al. 2004, 2008b; Welch et al. 2007). Cook et al. (2014) injected a dummy version of this cylindrical AT (same dimensions and weight as above) into juvenile Chinook Salmon (66–107 mm TL; transmitter burden = 1.5–7.3%) and held them in a laboratory for 21 d. Those authors found that inserting an AT into juvenile salmon via injection with a bevel-down method (i.e., with the bevel open toward the abdomen of the fish) using no rotation (i.e., inserted into the fish until approximately one-third of the bevel was inside the fish) resulted in high transmitter retention and survival rates for fish as small as 76 mm. This technique did not involve the use of sutures and resulted in rapid tagging procedures (Cook et al. 2014), which could reduce stress and handling time for the fish. However, Cook et al. (2014) recommended evaluating a greater number of individuals over a longer study duration to determine more robust size thresholds for this injection technique.

We followed suggestions by Cook et al. (2014) of injecting ATs into a greater number of fish (those authors examined 208 fish) over an extended holding period (their holding period was 21 d). Additionally, we examined two transmitter treatments using the bevel-down injection technique (their study used one transmitter treatment). By using a greater number of fish per treatment, we were able to perform more robust statistical analyses for determining a minimum size threshold. Our use of a longer study period also allowed for better analyses of postinjection behavioral responses (e.g., transmitter expulsion) for the duration of the transmitter's expected battery life. Therefore, our goal for this study was to evaluate response variables (mortality, transmitter expulsion rate, growth, and wound healing) for juvenile Chinook Salmon (n = 700) that were AT-injected by using the bevel-down method and were held for 60 d. We also sought to identify a minimum size threshold above which juvenile Chinook Salmon can be injected with micro ATs; this would allow us to determine the initial size at which the ATs no longer impact fish mortality, transmitter expulsion rates, growth, and wound healing—or at least to identify the initial size at which such effects are minimized.

METHODS

Fish acquisition and holding.—Juvenile Chinook Salmon were obtained in October 2012 as eyed eggs from Leavenworth National Fish Hatchery (U.S. Fish and Wildlife Service). The eggs were hatched and the juveniles were reared in 197-L raceways (300 cm long × 30 cm wide × 30 cm deep) at the Pacific Northwest National Laboratory (PNNL) Aquatic Research Laboratory (Richland, Washington) until they reached approximately 2–3 g in weight. All fish were reared in 650-L circular tanks (122 cm in diameter × 91 cm deep) supplied with sand-filtered, ultraviolet-treated, flow-through (flow set to 22 L/min) water from the Columbia River. Tank temperatures were maintained at 17.2 ± 0.53°C (mean ± SD), and the dissolved oxygen (DO) concentration was maintained between 8 and 10 mg/L. We used fluorescent lighting to simulate a natural photoperiod for fish. Fish that were selected for experimentation were separated into ten 189-L, semi-square tanks (64 cm long × 64 cm wide × 56 cm deep) to minimize the chance of losing all study fish to a disease outbreak; the 189-L tanks were set at the same temperature and DO levels as the 650-L circular tanks. During rearing and experimentation, fish were fed Bio Vita fry pellets (Bio-Oregon, Longview, Washington) once daily to satiation except that feed was withheld for 24 h prior to surgery.

Surgical protocols.—Study fish were originally tagged on July 23–25, 2013. However, a disease outbreak occurred during the first few weeks of experimentation. This required the replacement of fish in 6 of the 10 tanks, while the other four tanks showed no indications of disease (i.e., no emaciation, no flashing, and no increase in mortality). Fish in the six replacement tanks were tagged on August 14–16, 2013.

Regardless of the initial tagging day, study fish were randomly distributed into four treatments to minimize potential bias in tagging: (1) AT treatment, (2) AT+PIT treatment, (3) marked control, and (4) unmarked (true) control. Fish in the AT treatment were injected with a nonfunctioning cylindrical AT (length = 15.1 mm; maximum diameter = 3.5 mm; weight in air = 0.2 g; weight in water = 0.1 g; volume = 0.1 mL) that housed a PIT tag for individual identification. Fish in the AT+PIT treatment were injected with a nonfunctioning cylindrical AT (without a PIT tag embedded inside; same dimensions and weight as the AT mentioned above) and received a separate PIT tag (length = 12.5 mm; diameter = 2.1 mm; weight in air = 0.1 g; Destron Technologies, St. Paul, Minnesota). Fish in the marked control group were injected with visible implant elastomer (VIE; Northwest Marine Technology [NMT], Shaw Island, Washington). Fish in the unmarked (true) control group did not receive any tags or marks. Field studies in the Pacific Northwest commonly use PIT tags to identify fish as they enter juvenile bypass systems at hydroelectric dams (McMichael et al. 2011) and to ensure that the fish are not diverted into the fish transport barges or trucks that are used to bypass the hydroelectric projects. These facilities can identify fish based on their PIT tags and can route them back into the river. Consequently, a treatment that included PIT tags (i.e., the AT+PIT treatment) was incorporated. Marked control fish were used to obtain a more precise metric of individual growth (Brown et al. 2010) while using a minimally invasive marking technique. To maintain similar transmitter burdens for fish in the two injected treatments, the AT treatment fish ranged in FL from 65 to 94 mm (n = 150; Table 1), while the AT+PIT treatment fish ranged in FL from 75 to 104 mm (n = 150; Table 1). For comparison to each of the tagged treatments, the marked and unmarked control groups ranged in FL from 65 to 104 mm (n = 200 per treatment; Table 1). For all treatments, 2-mm size-bins (e.g., 65–66, 67–68, . . . , 103–104 mm) were used during tagging. This fine-scale resolution of the data was necessary to (1) ensure that a continuous range of fish sizes was tagged, (2) ensure that equivalent numbers of fish sizes were present in each holding tank, and (3) increase our likelihood of determining a minimum size threshold (i.e., using continuous data across the size range). Thus, we used a total of 700 fish for the study; this number of fish was also chosen to account for any potential mortality or expelled transmitters due to the long study duration (60 d) while maintaining adequate sample sizes to ensure that robust statistical analyses could still be performed. Each of the 10 study tanks held 70 fish from all four treatments and represented the range of sizes specified above for each treatment group.

As another measure to minimize bias, transmitter injections for the AT or AT+PIT treatments were performed by one person, and the VIE for the marked controls was injected by another person. Prior to surgery, fish were anesthetized with tricaine methanesulfonate (MS-222; 80 mg/L) buffered with sodium bicarbonate (80 mg/L), which acts as a base to balance the acidity of MS-222 (Carter et al. 2011). When the fish were anesthetized to stage 4 (defined by a complete loss of muscle tone, loss of equilibrium, and loss of spinal reflexes while a slow but steady opercular rate is maintained; Summerfelt and Smith 1990), the FL, weight (WT, g), and VIE codes were recorded. Transmitters for fish in the AT and AT+PIT treatments were injected into the coelom by using a bevel-down technique as described by Cook et al. (2014). Needles were 8 gauge, 70 mm long, with a 20-mm hub and a 15-mm-long, beveled vet point (Cook et al. 2014; Vita Needle Company, Needham, Massachusetts). Injections occurred approximately where the tip of the pectoral fin lies against the body and 2–3 mm above the linea alba (midline; toward the dorsal fin). Due to the shorter peritoneal cavity of small fish, the injection location had to be moved closer to the base of the pectoral fin in small (<75-mm FL) individuals (i.e., was more anterior than the injection location on larger fish). The open bevel of the needle faced the fish's abdomen and was inserted at approximately a 45° angle until there was a break in the peritoneum. After insertion into the peritoneal cavity, the needle angle was decreased to about 15° and was inserted until approximately one-third of the bevel was inside the fish, thus minimizing wound size. Because PIT tags are smaller than ATs, the PIT

TABLE 1. Initial FL, weight (WT), and transmitter burden for Chinook Salmon on the day of tagging (AT = fish injected with a cylindrical micro acoustic transmitter; AT+PIT = fish injected with an AT and a PIT tag; marked = fish injected with visible implant elastomer; unmarked = true control).

		Initial FL (mm)		Initial WT (g)		Transmitter burden (%)	
Treatment	*n*	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
AT	150	79 ± 8	65–94	5.2 ± 1.7	2.4–9.4	4.6 ± 1.6	2.2–8.7
AT+PIT	150	90 ± 8	75–104	7.5 ± 2.1	3.6–12.7	4.7 ± 1.4	2.5–8.9
Marked	200	84 ± 11	65–104	6.5 ± 2.5	2.4–12.8		
Unmarked	200	85 ± 11	65–104	6.5 ± 2.6	2.1–12.9		

tag was inserted prior to the AT to minimize the potential for PIT-tag loss in the AT+PIT treatment. The injection process took approximately 30 s, regardless of treatment. Fish in the marked control were injected with fluorescent VIE in three positions: (1) at the base of the dorsal fin, (2) in the adipose tissue behind the right eye, and (3) in the adipose tissue behind the left eye. The VIE was injected by using a handheld, 0.3-mL tuberculin syringe (29-gauge needle) coupled with a manual injector (NMT); the VIE injection process took about 70 s.

After injections, fish were photographed (AT and AT+PIT treatments) or mock-photographed (marked and unmarked controls). During real or mock photography (mean time = 61 s for injected fish, 36 s for controls), fish were placed ventral side up on a foam-rubber board covered in Fish Protector (Kordon LLC, Hayward, California; Harnish et al. 2011) and were supplied with fresh river water through tubing. For individuals that were injected with transmitters, a camera-enabled microscope (0.65× magnification; Stemi 2000-CS; Carl Zeiss Meditec AG, Jena, Germany) was used to take pictures for wound area analysis. The wound area was outlined and quantified by using imaging software (Image-Pro Plus and Image-Pro Analyzer version 7.0.1; Media Cybernetics, Bethesda, Maryland), and the microscope was calibrated at the beginning of each day. With the fish on the same plane as a ruler, the imaging software was used to outline the area of the wound during photograph evaluations. Determination of wound area was performed in real-time immediately after tagging and on each evaluation day. One observer looked at the fish through the microscope; to reduce wound area imaging bias, the same person was used as the observer for each evaluation day. A second observer outlined the wound area on the computer with guidance from the observer looking through the microscope. The total area was then calculated with Image-Pro Plus. To reduce bias in handling time, marked and unmarked fish were mock-photographed by resting the fish on the foam-rubber board. All fish were allowed to recover in buckets of aerated water until they reached equilibrium (~3 min). They were then transferred back into holding tanks (supplied with the same flow-through water source, temperature, and DO concentration as presurgery) until weekly evaluations commenced.

Evaluations.—Evaluations occurred on the day of injection (initial values) and once per week for 4 weeks postsurgery (days 7, 14, 21, and 28). We chose this frequency to better link the progression of FL, WT, and wound healing analyses with initial FL and potential transmitter effects. After the evaluation on day 28, the fish were held without evaluations until the end of the study (day 60) to minimize stress from additional handling. The 28-d posttagging threshold was chosen because most fish have recovered from surgery and their wounds are typically healed by that time (Deters et al. 2012). Fish were held for an additional 32 d (i.e., until day 60) to evaluate transmitter retention for the duration of the AT's expected battery life. On evaluation days, fish were anesthetized by use of MS-222 (80 mg/L) buffered with sodium bicarbonate (80 mg/L); the treatment, FL, and WT were then recorded, and transmitter presence was confirmed. Wound area was also recorded, and fish were supplied with a maintenance dose of buffered MS-222 (40 mg/L) during imaging analysis of the wound area. If the transmitter caused the wound to reopen, the entire area of the wound (including the exposed transmitter) was measured. Similar to the procedures used on the initial tagging date, marked and unmarked controls were mock-photographed and handled in a manner similar to that used for AT- and AT+PIT-injected fish. After the evaluation, fish were returned to a "new" tank (set at the same environmental conditions experienced by the fish before evaluation) and were held there until the next evaluation date; this was done to reduce tank effects. There was a rotation of 12 tanks for the 10 groups (i.e., fish resided in 5 different tanks over the study duration), and empty tanks were cleaned prior to use. Tanks were observed daily for dead fish and expelled transmitters. If a fish died, it was removed from the tank, and the treatment, FL, WT, and date were recorded. Transmitters that were found lying on the bottom of the tanks were removed with a telescoping magnetic pointer, and the treatment and date were recorded. Fish that had expelled their transmitters remained in the tanks until the next evaluation date, when they were removed from the study population and euthanized. After 60 d, all fish were euthanized with a 250-mg/L overdose of MS-222 and were evaluated via the same procedures used on previous evaluation days.

Statistical analyses.—Two null hypotheses were analyzed: (1) no difference in survival, FL growth, or WT gain for AT-

injected fish or AT+PIT-injected fish in comparison with marked controls; and (2) no difference in the probability of transmitter retention or wound area among the continuous size range of AT-injected fish or AT+PIT-injected fish (the two groups were considered separately). Categorical blocks included tank and treatment; fish were held in 10 tanks with each of the four treatments represented. Unmarked controls were only used in the analysis of overall mortality with treatment, as they were not tracked individually through the study. Statistical analyses were performed separately for each evaluation day; this was done to determine the size at which the transmitter treatments produced FL growth or WT that was not significantly different from that of the control group. To obtain greater statistical power, we used all fish with an initial tagging day adjustment instead of splitting the fish into smaller groups (size-bins) for analysis. Fish that died (including those that died as a result of jumping out of the tank) or expelled a transmitter were not included in the analyses of later evaluations. Initial analysis for tank effects involved the use of a generalized linear model (GLM; binomial distributions and logit link). Chi-square tests on the GLM examined among-tank and among-treatment differences in the probability of mortality and the probability of transmitter expulsion. Probability of transmitter expulsion was analyzed in relation to FL by using a logistic regression with a categorical response involving two possible outcomes (i.e., transmitter expulsion or transmitter retention).

We used GLM regression (Gaussian link; normal error distribution) to examine treatment effects on the dependent variables of FL growth, WT gain, and wound area after controlling for independent variables of initial FL, initial WT, and the initial FL (or initial WT) × treatment interaction, if significant. The FL growth and WT gain of each injection treatment (AT or AT +PIT) were compared with the marked control by using analysis of deviance (ANODEV). All stepwise models were built in the following order: tank effect, initial FL or WT, treatment (control or injected), and interactions. The null hypothesis was that transmitter expulsion, FL growth, or WT gain was not significantly affected by the tested variables.

To determine whether a minimum size threshold or "breakpoint" existed for each treatment, further analysis applied two types of spline regression with a single spline point or knot (e.g., the point at which the FL growth, WT gain, or wound area value abruptly shifted in relationship to the initial variable). This analysis was performed to assess whether transmitter effects on dependent variables changed for fish of different initial FL or initial WT (i.e., the independent variables). The knot was selected within the range of initial FLs or initial WTs, and the location that produced the highest R^2 value for the spline was deemed the best fit. Because spline regression will always produce an improved or equal R^2 value relative to that from an ordinary linear regression, an F-test was used to determine whether the spline point significantly improved model fit. The first spline used a single line that was "hinged" at the knot location; this was used on each individual treatment to characterize the initial FL or initial WT response. The second spline used two lines coming together to the knot, joining to a single, common line beyond that point. This analysis separately compared (1) the FL growth or WT gain from each injection treatment to that of the marked control, and (2) each injection treatment to its ordinary linear regression line to determine whether the treatment effect was significantly different for larger control fish (i.e., greater initial FL or WT). All statistical analyses were performed in R software (R Core Development Team 2014), with the significance level α set at 0.05.

RESULTS

Acoustic Transmitter-Injected Fish

In the comparison of AT-injected fish and marked control fish within the same size range, no tank effect ($P > 0.05$) was observed in the final ANODEV models for mortality on any of the five evaluation days. By day 60, 18 AT-injected fish had died and 57 AT-injected fish had expelled their transmitters, thus leaving only half ($n = 75$) of the fish alive and bearing transmitters.

Mortality was significantly higher among AT-injected fish than among marked control fish on all evaluation days ($P = 0.001$ for day 7; $P < 0.0001$ for days 14, 21, 28, and 60). Mortality was greater in smaller fish (initial FL = 65–88 mm; transmitter burden = 2.8–8.7%; Table 2). When comparing mortality between AT-injected fish and marked controls, there was a significant FL × treatment interaction for four of the five evaluation days ($P = 0.03$ for day 14; $P = 0.02$ for days 21, 28, and 60). This indicates that the survival of AT-injected fish differed due to their FLs on the initial tagging day and was not solely attributable to the presence of the transmitter.

There was no significant difference ($P = 1.00$) in FL on the initial tagging day between AT-injected fish and marked individuals. However, the GLM regression analysis detected significant initial FL and treatment effects on FL growth for all evaluation days (Table 3). A significant FL × treatment interaction effect on FL growth was detected for days 14, 21, and 28 (Table 3). As the initial FL increased, the difference in the regression slopes for marked controls and AT-injected fish decreased. When spline regression analyses were performed for those evaluation days, we found that the spline point (knot) was located near the maximum initial FL of fish tested (91.9 mm); therefore, two individual regression lines resulted in a better fit. This indicates that the difference in FL growth between AT-injected fish and marked controls increased with decreasing size of AT fish.

We found no significant difference ($P = 0.99$) between AT-injected fish and marked individuals in terms of WT on the initial tagging day. Across all five evaluation days, the GLM regression analysis identified a significant effect of initial WT on WT gain for AT-injected fish relative to marked controls and a significant effect of treatment (Table 4). A significant initial WT × treatment interaction effect was detected for day 14 but

TABLE 2. Initial FL, mortality, transmitter expulsion, and mean transmitter burden (range in parentheses) for juvenile Chinook Salmon in each treatment and each 5-mm size-bin. Mortality rate (%) and expulsion rate (%) columns represent the overall percentages (for example, 6 AT-injected fish in the 65–69-mm size-bin died out of a total of 150 AT-injected fish, resulting in 4.0% overall mortality). The FLs are measurements from the initial tagging day; mortality (*n*), transmitter expulsion (*n*), and mean transmitter burden (%) are for individuals at 60 d postinjection. Treatments included fish that were injected with a cylindrical micro-AT; fish that were injected with an AT and a PIT tag (AT+PIT); fish that were injected with visible implant elastomer (marked control); and a true control group (unmarked). Data for unmarked controls are assigned to the FL recorded on the day of mortality, as those fish were not tracked individually.

Initial FL (mm)	*n*	Mortality (*n*)	Mortality rate (%)	Expelled transmitters (*n*)	Expulsion rate (%)	Mean transmitter burden (%)
AT treatment						
65–69	24	6	4.0	17	11.3	7.2 (5.7–8.7)
70–74	26	1	0.7	20	13.3	5.8 (5.1–6.8)
75–79	24	5	3.3	14	9.3	4.9 (3.9–5.7)
80–84	26	4	2.7	3	2.0	4.0 (3.3–4.3)
85–89	26	2	1.3	3	2.0	3.1 (2.8–3.6)
90–94	24	0	0.0	0	0.0	2.7 (2.2–3.1)
AT+PIT treatment						
75–79	25	4	2.7	16	10.7	7.1 (6.3–8.9)
80–84	25	2	1.3	5	3.3	5.9 (5.1–6.6)
85–89	26	4	2.7	6	4.0	4.8 (4.2–5.6)
90–94	24	0	0.0	2	1.3	4.0 (3.4–4.6)
95–99	27	0	0.0	2	1.3	3.4 (2.9–3.8)
100–104	23	1	0.7	1	0.7	2.9 (2.5–3.5)
Marked control						
65–69	29	1	0.7			
70–74	21	0	0.0			
75–79	25	1	0.7			
80–84	25	0	0.0			
85–89	25	2	1.3			
90–94	25	0	0.0			
95–99	27	1	0.7			
100–104	23	0	0.0			
Unmarked control						
65–69	23	0	0.0			
70–74	27	2	1.3			
75–79	24	0	0.0			
80–84	26	2	1.3			
85–89	23	0	0.0			
90–94	27	0	0.0			
95–99	25	1	0.7			
100–104	25	1	0.7			

not for any of the other evaluation days (Table 4). Marked controls had a significantly greater WT gain than AT-injected fish; however, further analyses using spline regression failed to define a clear initial WT for establishing a minimum size threshold. This is because the knot location was located near the maximum WT of AT fish tested (8.8 g); therefore, two individual lines resulted in a better fit. This finding indicates that the difference in WT gain between AT-injected and marked controls increased with decreasing size of AT-injected fish.

By day 60, 57 AT-injected individuals had expelled their transmitters (Table 2), likely through the wound opening. The majority of expulsions occurred among 79-mm and smaller fish (transmitter burden $\leq$ 8.7%); however, the largest AT-injected fish to expel a transmitter was 89 mm FL (mean transmitter burden = 3.0%; Table 2). There was a significant effect of initial FL on the probability of transmitter expulsion for AT-injected fish across all evaluation days ($P < 0.0001$).

Spline regression analysis indicated a significant effect of initial FL on wound area for AT-injected fish. Size-related thresholds were found in the relationship between initial FL of AT fish and the wound area on day 14 (72.0 mm FL; $P < 0.0001$), day 21 (73.1 mm FL; $P = 0.02$), and day 60 (80.0 mm

TABLE 3. Analysis of deviance results for FL growth (mean ± SD) of juvenile Chinook Salmon in three treatment groups (AT = fish injected with a cylindrical micro acoustic transmitter; AT+PIT = fish injected with an AT and a PIT tag; marked = control fish injected with visible implant elastomer). Marked controls were compared separately to fish in the AT treatment and the AT+PIT treatment. A *P*-value less than 0.05 (denoted by an asterisk) indicates a significant effect of initial FL, treatment, or the initial FL × treatment interaction for specific evaluation days.

Evaluation day	Treatment	FL growth (mm)	Initial FL P (>F)	Treatment P (>F)	FL × treatment interaction P (>F)
7	AT	0.2 ± 1.0	0.006*	<0.0001*	0.14
	Marked	1.2 ± 1.1			
14	AT	1.6 ± 1.8	<0.0001*	<0.0001*	0.004*
	Marked	4.1 ± 1.9			
21	AT	5.0 ± 3.4	<0.0001*	<0.0001*	0.007*
	Marked	8.5 ± 3.0			
28	AT	8.9 ± 4.2	0.001*	<0.0001*	0.011*
	Marked	12.6 ± 3.7			
60	AT	29.6 ± 6.6	<0.0001*	0.001*	0.19
	Marked	32.9 ± 7.0			
7	AT+PIT	0.2 ± 1.0	0.47	<0.0001*	0.008*
	Marked	1.2 ± 1.1			
14	AT+PIT	2.3 ± 1.6	0.57	<0.0001*	0.0004*
	Marked	4.1 ± 1.9			
21	AT+PIT	5.9 ± 3.0	0.02*	<0.0001*	0.07
	Marked	8.5 ± 3.0			
28	AT+PIT	9.5 ± 3.8	0.02*	<0.0001*	0.1
	Marked	12.6 ± 3.7			
60	AT+PIT	29.1 ± 6.3	0.002*	0.01*	0.6
	Marked	32.9 ± 7.0			

FL; $P < 0.0001$). However, on days 14, 21, and 60, the spline knot was located near the smallest FL of fish tested (actual FL = 69.0 mm for days 14, 21, and 60). As such, ordinary linear regression provided a better fit than a spline regression, and no minimum size threshold could be determined for those days.

Acoustic Transmitter plus Passive Integrated Transponder-Injected Fish

In the comparison of AT+PIT-injected fish and similarly sized marked controls, no tank effect ($P > 0.05$) was observed in the final ANODEV models for mortality on any of the five evaluation days. By day 60, 11 AT+PIT-injected fish had died and 32 fish had expelled their transmitters, thus leaving about two-thirds ($n = 107$) of the AT+PIT-injected fish alive and bearing transmitters.

Mortality was significantly higher among AT+PIT-injected fish than among marked controls on all evaluation days (day 7: $P = 0.04$; day 14: $P = 0.003$; day 21: $P = 0.005$; day 28: $P = 0.005$; day 60: $P = 0.002$). For the AT+PIT treatment, initial FLs of the fish that died ranged from 76 to 101 mm (transmitter burden = 2.8–8.9%; Table 2). Although the majority of the mortality occurred in 89-mm and smaller fish, one large fish (101 mm FL) died at 9 d posttagging. In comparing the mortality of AT+PIT-injected fish to that of marked controls, the initial FL × treatment interaction effect was not significant for any evaluation day ($P > 0.50$). However, the marked control fish that died had smaller initial FLs (survival increased for larger marked control fish) than the mortalities of AT+PIT-injected fish.

There was no significant difference ($P = 0.97$) in FL between AT+PIT-injected fish and marked control fish on the initial tagging day. However, the GLM regression analysis detected significant effects of initial FL on FL growth for days 21, 28, and 60 (Table 3). Across all evaluation days, there was a significant treatment effect on FL growth (Table 3); marked controls had significantly greater FL growth than AT+PIT-tagged fish. On days 7 and 14, there was a significant interaction effect; however, as initial FL increased, the differences in FL growth between marked controls and AT+PIT-injected fish decreased, and by day 21 the differences were not significant (Table 3). After further examination using spline regression analyses, we identified the knot as being located near the maximum initial FL tested (knot = 101.9 mm FL; maximum for AT+PIT-injected fish = 104 mm FL); therefore, two individual ordinary linear regression lines resulted in a better fit.

We found no significant difference ($P = 0.97$) when comparing the WT of AT+PIT-injected fish to that of marked control individuals on the initial tagging day. The GLM regression analysis identified (1) a significant effect

TABLE 4. Analysis of deviance results for weight (WT) gain (mean ± SD) of juvenile Chinook Salmon in three treatment groups (AT = fish injected with a cylindrical micro acoustic transmitter; AT+PIT = fish injected with an AT and a PIT tag; marked = control fish injected with visible implant elastomer). Marked controls were compared separately to fish in the AT treatment and the AT+PIT treatment. A *P*-value less than 0.05 (denoted by an asterisk) indicates a significant effect of initial WT, treatment, or the initial WT × treatment interaction for specific evaluation days.

Evaluation day	Treatment	WT gain (g)	Initial WT P (>F)	Treatment P (>F)	WT × treatment interaction P (>F)
7	AT	0.10 ± 0.4	<0.0001*	<0.0001*	0.06
	Marked	0.6 ± 0.4			
14	AT	0.8 ± 0.9	<0.0001*	<0.0001*	0.045*
	Marked	1.9 ± 0.8			
21	AT	2.0 ± 1.4	<0.0001*	<0.0001*	0.06
	Marked	3.3 ± 1.9			
28	AT	3.5 ± 1.7	<0.0001*	<0.0001*	0.24
	Marked	4.7 ± 1.7			
60	AT	13.5 ± 3.9	<0.0001*	0.005*	0.35
	Marked	14.9 ± 4.2			
7	AT+PIT	0.2 ± 0.4	<0.0001*	<0.0001*	0.06
	Marked	0.6 ± 0.4			
14	AT+PIT	1.3 ± 1.0	<0.0001*	<0.0001*	0.11
	Marked	1.9 ± 0.8			
21	AT+PIT	2.8 ± 1.4	<0.0001*	<0.0001*	0.21
	Marked	3.3 ± 1.9			
28	AT+PIT	4.3 ± 1.8	<0.0001*	<0.0001*	0.37
	Marked	4.7 ± 1.7			
60	AT+PIT	15.2 ± 4.0	<0.0001*	0.009*	0.92
	Marked	14.9 ± 4.2			

of initial WT on WT gain and (2) a significant treatment effect on WT gain across all evaluation days when comparing AT+PIT-injected fish with marked controls (Table 4). However, the lack of a significant interaction for any of the evaluation days (Table 4) indicated that fish experienced similar WT gain regardless of treatment. Follow-up spline regression analysis was unable to identify a significant minimum size threshold for AT+PIT-injected fish and marked controls. The knot for the spline regression analyses was located near the maximum WT of AT+PIT fish tested (knot = 12.1 g; maximum WT = 12.7 g); therefore, two individual regression lines resulted in a better fit.

By day 60, transmitters had been expelled by 32 AT+PIT-injected fish across a range of sizes (initial FL = 75–101 mm; transmitter burden = 2.8–8.9%; Table 2); the transmitters likely were expelled through the wound opening. Transmitter expulsion occurred at a higher rate in small fish (≤89 mm FL) than in the large fish, although one large individual (101 mm FL) expelled its PIT tag at 27 d posttagging. That fish did not expel the AT, but the PIT expulsion still resulted in a significant effect of initial FL on the probability of transmitter expulsion for AT+PIT-injected fish across all evaluation days (day 7: $P = 0.01$; all other days: $P < 0.0001$).

For AT+PIT-tagged individuals, spline regression analysis indicated a significant effect of initial FL on wound area (Table 5). A size-related threshold was found in the relationship between initial FL and wound area on day 7 (threshold = 85.1 mm FL; mean transmitter burden = 5.3%; Figure 1). Thus, in the AT+PIT treatment by day 7, wound areas of fish smaller than 85.1 mm FL were significantly greater than the wound areas of fish exceeding 85.1 mm FL. Another size-related threshold was detected for day 60 (84.0 mm FL; Table 5). However, on day 60, some AT+PIT treatment fish within the initial FL range of 76–89 mm had wound areas between 0.03 and 0.09 mm^2 (Table 5) and one fish exhibited a 0.76-mm^2 wound area; wounds on the remaining fish were completely healed. The close grouping of these individuals created a significant knot at 84.0 mm FL. Comparison of the R^2 for the spline regression versus ordinary linear regression indicated that the improvement was minimal and should be discounted.

DISCUSSION

We examined an injection technique for implanting new micro ATs that were specifically designed for implantation via injection. This technique was implemented because implanting a tag by injection into a fish's abdomen can be faster than

TABLE 5. Relationships between initial FL and wound area compared by using ordinary linear regression and spline regression for juvenile Chinook Salmon that were injected with a cylindrical micro acoustic transmitter and a PIT tag (AT+PIT treatment) or that were marked with visible implant elastomer (marked control group). For each evaluation day, the goodness of fit for the models (R^2) is indicated. For ordinary linear regressions, it was determined whether the models were significantly ($P < 0.05$) different from a straight line. For spline regressions, a P-value less than 0.05 for either side of the knot location (e.g., the point at which a size threshold was observed) indicated that the spline regression significantly improved model fit in comparison with a linear regression. Asterisks denote significant knot locations.

	Ordinary linear regression		Spline regression			
Evaluation day	R^2	P (>F)	R^2	Knot location (mm FL)	Left side	Right side
7	0.38	<0.0001	0.45	85.1*	$P(F_{91,\ 45} > 2.29) = 0.001$	$P(F_{45,\ 91} > 11.18) < 0.0001$
14	0.14	<0.0001	0.15	93.0	$P(F_{57,\ 61} > 0.50) = 0.99$	$P(F_{61,\ 57} > 3.37) < 0.0001$
21	0.11	0.0003	0.15	96.0	$P(F_{44,\ 66} > 0.79) = 0.79$	$P(F_{66,\ 44} > 4.75) < 0.0001$
28	0.15	<0.0001	0.16	89.0	$P(F_{71,\ 36} > 0.20) = 1.00$	$P(F_{36,\ 71} > 11.75) < 0.0001$
60	0.03	0.1030	0.04	84.0	$P(F_{88,\ 17} > 11.59) < 0.0001$	$P(F_{17,\ 88} > 0.30) = 0.99$

using a surgery, which requires an incision, hand insertion of a transmitter, and sutures (Cook et al. 2014; Deng et al. 2015). Our study was a follow-up to the work of Cook et al. (2014), but we examined a greater number of injected fish over a longer study duration, and we used two transmitter treatments to better understand the initial fish size at which transmitter effects on mortality, retention, growth, and wound healing are no longer present. Injection of an AT or an AT+PIT had a greater effect on smaller juvenile Chinook Salmon than on larger fish. The ability to tag smaller fish has long been a goal of researchers using transmitters in several species (Winter 1983; Brown et al. 1999, 2010, 2013; Smircich and Kelly 2014; Snobl et al. 2015). Among AT- or AT+PIT-injected fish, those with smaller initial FLs were more likely to die than larger fish, probably due to the small size of fish tagged in this study. There was a significant FL × treatment interaction effect on the mortality of AT-injected fish in comparison with marked controls. Larger AT-injected individuals had a higher rate of survival than smaller individuals; however, this was more likely due to initial fish size than to the presence of the transmitter itself. Interestingly, two of the mortalities in the marked control group had larger initial FLs (e.g., survival declined for larger marked control fish), resulting in a decrease in expected survival for larger marked fish. This may have caused the significant interaction effect on mortality for AT-injected individuals and marked controls.

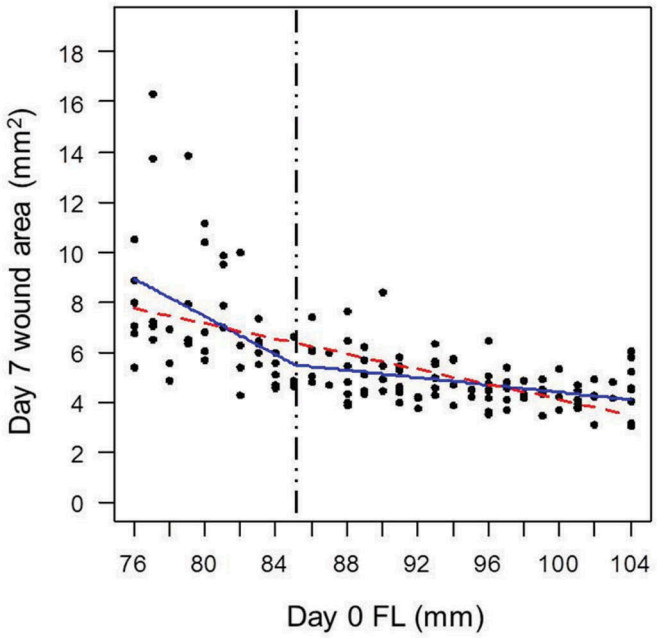

FIGURE 1. Example of a spline regression graph comparing initial FL to day-7 wound area for juvenile Chinook Salmon that were injected with a cylindrical micro acoustic transmitter and a PIT tag (AT+PIT treatment). The spline regression (blue solid line) and linear regression (red dashed line) were plotted using the same data because marked controls were not available (i.e., they did not have a wound area). The spline break (located at 85.1 mm FL; vertical dashed–dotted line) is significant ($P = 0.001$) and resulted from a dramatic increase in wound area for smaller fish.

Other studies examining Chinook Salmon (Brown et al. 2006) and steelhead *O. mykiss* (Welch et al. 2007) also found that survival was strongly associated with fish size. Survival in those studies was lower among tagged fish than among controls, similar to the present results. Notably, most of the mortality in our study occurred within the first 21 d posttagging; likewise, most of the mortality of juvenile Chinook Salmon in the Brown et al. (2006) and Cook et al. (2014) studies also occurred within 21 d. Regardless of the reason, marked control fish had better survival rates than fish in the AT and AT+PIT treatments, and the effect of injection on mortality was more prominent in smaller AT- and AT+PIT-injected fish than in larger fish.

Although the majority of the mortalities were observed in the injected treatments, marked and unmarked control fish were also subject to mortality, suggesting that some of the mortality in the

AT and AT+PIT treatments was caused by factors other than simply the transmitter effect. A possible cause of the observed mortality is the initial condition of the Chinook Salmon, which were hatchery raised as opposed to wild stock. Six tanks of fish contracted a disease, and all of the fish we used were from the same source population, indicating that the fish were not in the best condition regardless of treatment. These factors may have contributed to mortality among AT+PIT-injected fish as large as 101 mm FL (mean transmitter burden = 3.0%) and among AT-injected fish as large at 89 mm FL (mean transmitter burden = 3.0%). Unmarked and marked control fish had shorter anesthetic exposures and shorter handling times than the treatment fish, yet there were still mortalities among larger marked control fish (95 mm FL on day 29). Previous work (Panther et al. 2011) also documented mortalities in juvenile Chinook Salmon belonging to a control group, but those fish were held at a warmer water temperature (20°C), and there was no significant difference in mortality between the controls and the fish that received surgically implanted transmitters (Panther et al. 2011). Those results differed from ours, but we used smaller fish and a larger size range (65–104 mm FL) than Panther et al. (2011; 95–121 mm FL). Cook et al. (2014) also documented mortality in juvenile Chinook Salmon belonging to marked and true control treatments, although mortality exhibited no significant difference among tagged and control treatments. The control treatments in the current study had lower overall mortality than either of the injected treatments, but the mortality that did occur among controls indicates that the transmitter effect was not the only factor contributing to mortalities among fish in the injection treatments.

By the end of our study, over one-third of the AT-injected fish and nearly 20% of the AT+PIT-tagged fish had expelled their transmitters (or PIT tags), and transmitter expulsion was more evident for smaller fish than for larger fish. Fish in the AT treatment group began to expel their transmitters at 1 d posttagging, and expulsion continued through 37 d posttagging. However, the AT-injected fish that expelled transmitters in the first 14 d posttagging were the smallest individuals (≤86 mm FL; transmitter burden = 3.5–8.7%). For the AT+PIT treatment, fish began to expel their transmitters at 2 d posttagging, with expulsions continuing through 50 d posttagging. The AT+PIT-injected fish that expelled transmitters during the first 14 d posttagging were also the smallest individuals (≤90 mm FL; transmitter burden = 4.0–8.9%). The rate of transmitter expulsion in our study was greater than that recorded for juvenile Chinook Salmon in the "bevel-down" injection treatment as reported by Cook et al. (2014); the expulsion rate in their study was 7.7% at 21 d postimplantation, although their sample sizes were smaller. The transmitter expulsion rate we observed may have been caused by the initial health of the fish, a lack of wound healing (closure), or the greater amount of weekly handling (more netting, air exposure, and anesthetic exposure, resulting in greater stress). We used four evaluation days in addition to the initial tagging day, whereas Cook et al. (2014) had only two evaluation days in addition to their initial tagging day. Furthermore, Cook et al. (2014) did not examine an AT+PIT treatment. However, in the present study, the only fish that expelled transmitters in the AT treatment were 89 mm FL or smaller, whereas the majority (~84%) of fish that expelled transmitters in the AT+PIT treatment were 89 mm FL or smaller.

We did not explicitly measure the body cavity length in our study fish, but transmitter expulsion could be explained by the limited body cavity length for the smaller tagged fish. The location of the injection point was moved anteriorly for small fish due to their smaller peritoneal cavity. Smaller body cavity length may have prevented the transmitter from completely fitting inside the peritoneal cavity, thereby causing the fish to expel the transmitter. This idea of limited body cavity length as an explanation for transmitter expulsion from small fish is similar to other studies that have also documented the expulsion of transmitters from the body cavities of juvenile salmonids (steelhead: Welch et al. 2007; Brown Trout *Salmo trutta*: Jepsen et al. 2008b; Chinook Salmon; Cook et al. 2014). In field studies, Larsen et al. (2013) found that the survival of PIT-tagged Atlantic Salmon was not impaired for fish larger than 103 mm FL. Similarly, Brown et al. (2013) found that the survival of AT+PIT-tagged subyearling Chinook Salmon was significantly impacted by FL: fish with larger FLs (>89 mm) had a higher probability of survival than smaller (80–89-mm) fish. However, compared with the current study, the fish studied by Brown et al. (2013) were larger overall (FL = 80–129 mm) and had a greater range of transmitter burden (2.5–15.1%). Regardless of the mechanism, this study shows that transmitter expulsion in the AT or AT+PIT treatment was significantly affected by the initial size of the fish. Although no minimum size thresholds were found, transmitter expulsion occurred less frequently among fish larger than 90 mm in both treatments.

Growth in FL and WT was also negatively influenced by transmitter injection in the AT and AT+PIT treatments relative to marked controls; this was more apparent in smaller injected fish than in larger fish. However, no minimum size threshold was identified for any of the evaluation days in either injection treatment. The reduced growth in AT- and AT+PIT-injected individuals could be a result of energy being allocated toward recovery from surgery or wound healing, potentially leading to reduced feeding and growth performance (Jepsen et al. 2008b; Wargo Rub et al. 2014). Wargo Rub et al. (2014) also suggested that actual space taken up by a transmitter may reduce feeding, thereby reducing growth as well. Jepsen et al. (2008b) reported that Brown Trout (150–290 mm) in the control had significantly greater specific growth rates than fish in the tagged treatments. However, the reduced growth observed by Jepsen et al. (2008b) did not affect the survival of tagged Brown Trout. Likewise, the transmitters' negative effect on growth of AT- and AT+PIT-injected fish in our study might not have influenced survival, as mortalities were also observed among the marked and unmarked control fish. For steelhead into which transmitters were surgically implanted, Welch et al. (2007; 110–130 mm

long; mean transmitter burden = 7.0%) also found that growth was reduced relative to that of controls, with greater evidence in smaller tagged fish than in larger tagged fish. This was similar to the FL and WT growth responses of fish in the present study. Analogous to the other responses measured here, marked control fish significantly grew in FL and gained significantly more WT than either of the tagged treatments, although this was less apparent in the larger tagged fish than in the smaller fish.

Among AT- and AT+PIT-injected fish, individuals with smaller initial FLs typically had a larger wound area than larger fish, and larger individuals experienced better wound healing (i.e., wound area decreased at a faster rate). Although this was the trend, no minimum size threshold was found for AT-injected fish, possibly due to the variability in wound area across the size range of tagged fish (65–94 mm FL) across evaluation days; however, we used larger sample sizes than other studies of transmitter effects (Welch et al. 2007; Jepsen et al. 2008b; Cook et al. 2014). For AT+PIT-tagged fish, a significant threshold (initial FL = 85.1 mm) was detected for wound area on day 7. An increase in wound area on day 7 compared with the initial wound area was also noted by Cook et al. (2014), who used similarly sized Chinook Salmon (66–108 mm FL). We observed the increase in wound area on day 7 for the AT and AT+PIT treatments, but wound area began to decrease for all fish by day 14 and decreased through the end of the study, irrespective of the initial FL measurement. Wound healing was expected after the initial swelling on days 7 and 14, and Cook et al. (2014) also noted a similar decrease in wound area through time. However, by the end of the Cook et al. (2014) study (21 d), wounds were not fully healed. In our study, almost all wounds were completely healed by day 60 for AT- and AT+PIT-injected individuals. This finding is similar to those of other wound healing studies, such as the study by Panther et al. (2011), who found that 99% of incisions were healed by 63 d posttagging in Chinook Salmon that were held at 20°C. Deters et al. (2010) also reported complete incision closure by 63 d posttagging for Chinook Salmon that were held at a mean temperature of 17°C. Even though wound areas were larger for the smaller fish belonging to the AT and AT+PIT treatments, wound area did not affect wound healing ability for fish that did not expel their transmitters by the end of the study.

Establishing a minimum size threshold at which to employ this injection technique is critical, as many organizations use size limits to determine the appropriate size at which fish can be tagged in field studies. For example, a common minimum size limit of 95 mm FL is used in tagging juvenile Chinook Salmon in the Columbia River basin (SPSC 2011). We did not identify a minimum size threshold for mortality or transmitter expulsion in the present study, but it should be noted that no mortality or expulsion was observed among AT-injected fish that were 90 mm or larger. For AT+PIT-tagged fish, the majority of mortalities and transmitter expulsions occurred in fish that were smaller than 90 mm. Mortality and transmitter expulsion among larger fish in the AT+PIT treatment may have been caused by factors in addition to the injection (e.g., weekly handling events, time of year [fish were beginning to smolt by the end of our study], fish stock, etc.), as mortality also occurred in larger fish belonging to the marked and unmarked treatments. Additionally, the transmitter burden was only 2.8%. This is well below the 6.7% burden that was found to negatively affect survival in Chinook Salmon (Brown et al. 2010), and it is within an appropriate range for our specific study objectives (Jepsen et al. 2004). However, future studies of transmitter effects should include a parallel treatment of fish that are not handled or evaluated until the end of the study; the responses of fish mortality, transmitter expulsion, growth, and wound healing in the absence of multiple handling events could then be determined. Many of the spline knot locations identified in this research were located at the minimum or maximum size of study fish used. Therefore, future studies may benefit from the inclusion of a wider range of fish, which would allow for better differentiation of the spline break. Additionally, comparative field studies should be performed to evaluate the effectiveness of the micro AT in migrating fish (similar to the work of Brown et al. 2013).

ACKNOWLEDGMENTS

We thank the U.S. Army Corps of Engineers Portland District for providing the funding for this research. We are also grateful to Leavenworth National Fish Hatchery (U.S. Fish and Wildlife Service) for providing Chinook Salmon eggs; Tim Linley (PNNL) for assistance in fish husbandry and laboratory use; and Makenzie Daniels, Kris Hand, Amanda Hanson, Brian Jeide, Rachelle Johnson, Jina Kim, Ryan Klett, Sadie Montgomery, Brett Pflugraph, Gabrielle Schuler, John Stephenson, and Lauren Stoot (PNNL) for assisting with data collection.

REFERENCES

Adams, N. S., D. W. Rondorf, S. D. Evans, and J. E. Kelly. 1998. Effects of surgically and gastrically implanted radio transmitters on growth and feeding behavior of juvenile Chinook Salmon. Transactions of the American Fisheries Society 127:128–136.

Brown, R. S., S. J. Cooke, W. G. Anderson, and R. S. McKinley. 1999. Evidence to challenge the “2% rule” for biotelemetry. North American Journal of Fisheries Management 19:867–871.

Brown, R. S., D. R. Geist, K. A. Deters, and A. Grassell. 2006. Effects of surgically implanted acoustic transmitters >2% of body mass on the swimming performance, survival and growth of juvenile Sockeye and Chinook salmon. Journal of Fish Biology 69:1626–1638.

Brown, R. S., R. A. Harnish, K. M. Carter, J. W. Boyd, K. A. Deters, and M. B. Eppard. 2010. An evaluation of the maximum tag burden for implantation of acoustic transmitters in juvenile Chinook Salmon. North American Journal of Fisheries Management 30:499–505.

Brown, R. S., E. W. Oldenburg, A. G. Seaburg, K. V. Cook, J. R. Skalski, M. B. Eppard, and K. A. Deters. 2013. Survival of seaward-migrating PIT and acoustic-tagged juvenile Chinook Salmon in the Snake and Columbia rivers: an evaluation of length-specific tagging effects. Animal Biotelemetry [online serial] 1:8.

Carter, K. M., C. M. Woodley, and R. S. Brown. 2011. A review of tricaine methanesulphonate for anesthesia of fish. Reviews in Fish Biology and Fisheries 21:51–59.

Chen, H., S. Cartmell, Q. Wang, T. Lozano, Z. D. Deng, H. Li, X. Chen, Y. Yuan, M. E. Gross, T. J. Carlson, and J. Xiao. 2014. Micro-battery development for juvenile salmon acoustic telemetry system applications. Scientific Reports 4:1–5.

Cook, K. V., R. S. Brown, Z. D. Deng, R. S. Klett, H. Li, A. G. Seaburg, and M. B. Eppard. 2014. A comparison of implantation methods for large PIT tags or injectable acoustic transmitters in juvenile Chinook Salmon. Fisheries Research 154:213–223.

Cooke, S. J., C. M. Woodley, M. B. Eppard, R. S. Brown, and J. L. Nielsen. 2011. Advancing the surgical implantation of electronic tags in freshwater and marine fish: a gap analysis and research agenda based on a review of trends in intracoelomic tagging effects studies. Reviews in Fish Biology and Fisheries 21:127–151.

Deng, Z. D., T. J. Carlson, H. Li, J. Xiao, M. J. Myjak, J. Lu, J. J. Martinez, C. M. Woodley, M. A. Weiland, and M. B. Eppard. 2015. An injectable acoustic transmitter for juvenile salmon. Scientific Reports 5(8111):1–6.

Deters, K. A., R. S. Brown, J. W. Boyd, M. B. Eppard, and A. G. Seaburg. 2012. Optimal suturing technique and number of sutures for surgical implantation of acoustic transmitters in juvenile salmonids. Transactions of the American Fisheries Society 141:1–10.

Deters, K. A., R. S. Brown, K. M. Carter, J. A. Boyd, and M. B. Eppard. 2010. Performance assessment of suture type in juvenile Chinook Salmon. Transactions of the American Fisheries Society 139:888–899.

Harnish, R. A., A. H. Colotelo, and R. S. Brown. 2011. A review of water conditioners for reduction of handling-related injury during surgical implantation of transmitters. Reviews in Fish Biology and Fisheries 21:43–49.

Jepsen, N., M. Christoffersen, and T. Munksgaard. 2008a. The level of predation used as indicator of tagging/handling effects. Fisheries Management and Ecology 15:365–368.

Jepsen, N., J. S. Mikkelsen, and A. Koed. 2008b. Effects of tag and suture type on survival and growth of Brown Trout with surgically implanted telemetry tags in the wild. Journal of Fish Biology 72:594–602.

Jepsen, N., C. Schreck, S. Clement, and E. Thorstad. 2004. A brief discussion of the 2% tag/body mass rule. Pages 255–259 *in* M. T. Spedicato, G. Marmulla, and G. Lembo, editors. Aquatic telemetry: advances and applications. Food and Agriculture Organization of the United Nations, COISPA Technology and Research, Rome.

Larsen, M. H., A. N. Thorn, C. Skov, and K. Aarestrup. 2013. Effects of passive integrated transponder tags on survival and growth of juvenile Atlantic Salmon *Salmo salar*. Animal Biotelemetry [online serial] 1:19.

McMichael, G. A., J. R. Skalski, and K. A. Deters. 2011. Survival of juvenile Chinook Salmon during barge transport. North American Journal of Fisheries Management 31:1187–1196.

Panther, J. L., R. S. Brown, G. L. Gaulke, K. A. Deters, C. M. Woodley, and M. B. Eppard. 2011. Influence of incision location on transmitter loss, healing, incision length, and suture retention of juvenile Chinook Salmon. Transactions of the American Fisheries Society 140:1492–1503.

Peven, C., A. Giorgi, J. R. Skalski, M. Langeslay, A. Grassell, S. Smith, T. Counihan, R. Perry, and S. Bickford. 2005. Guidelines and suggested protocols for conducting, analyzing, and reporting juvenile salmonid survival studies in the Columbia River basin. University of Washington, School of Aquatic and Fishery Sciences, Columbia Basin Research, Seattle.

PTSC (PIT Tag Steering Committee). 2014. PIT tag marking procedures manual. Pacific States Marine Fisheries Commission, Portland, Oregon.

R Core Development Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Rechisky, E. L., D. W. Welch, A. D. Porter, M. C. Jacobs-Scott, and P. M. Winchell. 2013. Influence of multiple dam passage on survival of juvenile Chinook Salmon in the Columbia River estuary and coastal ocean. Proceedings of the National Academy of Sciences of the USA 110:6883–6888.

Skalski, J. R., R. L. Townsend, T. W. Steig, P. A. Nealson, and A. Grassell. 2006. Survival of yearling Chinook, Sockeye salmon, and steelhead smolts through Rocky Reach and Rock Island projects in 2005. University of Washington, School of Aquatic and Fishery Sciences, Columbia Basin Research, Seattle.

Smircich, M. G., and J. T. Kelly. 2014. Extending the 2% rule: the effects of heavy internal tags on stress physiology, swimming performance, and growth in Brook Trout. Animal Biotelemetry [online serial] 2:16.

Snobl, Z. R., R. P. Koenigs, and R. M. Bruch. 2015. Do tags exceeding 2% of total body weight impair Lake Sturgeon movement? North American Journal of Fisheries Management 35:880–884.

Summerfelt, R. C., and L. S. Smith. 1990. Anesthesia, surgery, and related techniques. Pages 213–272 *in* C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

SPSC (Surgical Protocols Steering Committee). 2011. Surgical protocols for implanting JSATS transmitters into juvenile salmonids for studies conducted for the U.S. Army Corps of Engineers. Prepared for the U.S. Army Corps of Engineers, Portland District, Portland, Oregon.

Walker, R. W., N. K. Ashton, R. S. Brown, S. A. Liss, A. H. Colotelo, B. V. Beirão, R. L. Townsend, Z. D. Deng, and M. B. Eppard. 2016. Effects of a novel acoustic transmitter on swimming performance and predator avoidance of juvenile Chinook Salmon: determination of a size threshold. Fisheries Research 176:48–54.

Wargo Rub, A. M., N. Jepsen, T. L. Liedtke, M. L. Moser, and E. P. S. Weber. 2014. Surgical tagging and telemetry methods in fisheries research: promoting veterinary and research collaboration. American Journal of Veterinary Research 75:402–416.

Welch, D. W., S. D. Batten, and B. R. Ward. 2007. Growth, survival, and tag retention of steelhead trout (*O. mykiss*) surgically implanted with dummy acoustic tags. Hydrobiologia 582:289–299.

Winter, J. D. 1983. Underwater biotelemetry. Pages 371–395 *in* L. A. Nielsen and D. L. Johnson, editors. Fisheries techniques. American Fisheries Society, Bethesda, Maryland.