Neil K. Ashton[1], **Stephanie A. Liss**[2], **Ricardo W. Walker**[3], **Richard S. Brown**, Pacific Northwest National Laboratory, 902 Battelle Blvd. PO Box 999, Richland, Washington 99354

Cheryl Klassen, Stephanie Backhouse, Manitoba Hydro, 19 Henlow Bay, Winnipeg, MB R3Y 1G4, Canada

Phil Bates, Idaho Power Company, 1221 W Idaho St, Boise, Idaho 83702

and

Richard L. Townsend, University of Washington, 1410 NE Campus Parkway, PO Box 355852, Seattle, Washington 98195

How Low Can You Go? Determining a Size Threshold for Implantation of a New Acoustic Transmitter in Age-0 White Sturgeon

Abstract

Telemetry studies are often used to investigate sturgeon habitat use and movement patterns; however, existing acoustic transmitters are generally too large to implant into age-0 sturgeon without harming the fish. Recent development of a miniaturized acoustic transmitter (cylindrical, 0.7 g in air, 24.2 mm long, 5.0 mm diameter) with up to 365 d battery life has the potential to advance our understanding of age-0 sturgeon ecology in rivers and lakes. Prior to use in field studies, it is essential to conduct experiments evaluating potential adverse transmitter effects on fish. We tested transmitter retention, fish survival, and growth of a broad size range of age-0 white sturgeon (*Acipenser transmontanus*; 158–277 mm fork length; 26–126 g; 0.6–2.6% transmitter burden) in an 84 d laboratory study, with an ultimate goal of determining a minimum size threshold of sturgeon that can be implanted with this acoustic transmitter. At 84 d post-implantation, transmitter retention and fish survival were 100%. Specific growth rates were reduced at 7 and 14 d post-implantation, resulting in minimum fork length thresholds of 250 and 171 mm, respectively. Juveniles implanted with transmitters regained their growth potential by 28 d post-implantation and no size differences were detected in comparisons with unmarked control fish. This study demonstrates the ability to implant small age-0 sturgeon with high transmitter retention and fish survival, and only minor growth effects. Use of new miniaturized acoustic transmitters may give researchers a means to address questions about young-of-the-year fish recruitment, ecological patterns, and potentially advance conservation management of sturgeon populations.

Keywords: behavior, juvenile sturgeon, movement, size-dependent effects, telemetry

Introduction

Many species of sturgeon (Family Acipenseridae) have experienced major population declines due to a combination of overfishing (either historical or ongoing), habitat degradation and fragmentation, exploitation, and inherent life-history challenges (e.g., slow rates of reproduction). Consequently, Acipenseridae are considered one of the most endangered groups of animals in the world according to the International Union for Conservation of Nature (Nelson et al. 2014). There has been substantial research on sturgeon movement patterns, habitat preferences, and assessments of year-class strength and natural recruitment (Neufeld and Rust 2009, Barth et al. 2011, Trested et al. 2011, Bates et al. 2014, McDougall et al. 2014a, Hondorp et al. 2015). However, many of these investigations have involved larger (> 300 mm total length) or older (> age-0) sturgeon capable of withstanding transmitter implantation or attachment; or, incorporated indirect and labor intensive telemetry methods (e.g., mark-recapture using tags with individual identification [ID]). Subsequently, local movements and habitat preferences are not well understood for juvenile sturgeon that are too small for transmitter implantation (Peterson et al. 2007, Parsley et al. 2008), particularly in deep

[1]Current Address: University of Idaho, 709 S. Deakin St, Moscow, Idaho 83844

[2]Author to whom correspondence should be addressed. Email: stephanie.liss@pnnl.gov

[3]Current Address: U.S. Army Corps of Engineers, 333 SW 1st Ave #200, Portland, Oregon 97204

riverine and lacustrine environments that are difficult to sample using mark-recapture techniques. Our lack of understanding age-0 sturgeon ecology presents challenges for conservation programs seeking to monitor fisheries and forecast productivity. The development of a very small acoustic transmitter with a transmitter life sufficient to follow fish through this early life history stage (i.e., 365 d) would greatly increase the resolution of studies evaluating ecological patterns of age-0 sturgeon (Johnson et al. 2014), and could be a valuable tool for large-scale assessments of juvenile population dynamics (McMichael et al. 2010, Skalski et al. 2014).

A novel, miniaturized acoustic transmitter (cylindrical, 0.7 g in air, 24.2 mm long, 5.0 mm diameter) has been developed for monitoring small age-0 sturgeon for up to 365 d at a 15-s ping rate set at a source level of 161 decibels (Lu et al. 2016). Before using the transmitters it is critical to perform laboratory studies to minimize uncertainty about the dependability of data collected in field studies (Cooke et al. 2011). Laboratory studies can identify implantation effects on transmitter retention, fish survival, and growth. This information can then be used to determine a minimum size threshold for implanting fish with acoustic transmitters in field studies and to minimize adverse effects either due to the transmitter or surgical procedure.

It is well-known that the implantation process (i.e., netting, handling, anesthesia, surgery) can adversely affect transmitter retention, survival, growth, or swimming performance of implanted fish (Adams et al. 1998, Lacroix et al. 2004, Zale et al. 2005, Welch et al. 2007). To minimize adverse transmitter effects, a commonly used guideline in telemetry studies has been to maintain a ≤ 2% transmitter burden (i.e., the weight of the transmitter relative to the weight of the fish) for implanted fish (Winter 1983). However, this can potentially introduce bias for estimates of behavior and survival by only implanting transmitters into larger individuals within a cohort (Skalski et al. 2006) in order to maintain a ≤ 2% transmitter burden. Other research has demonstrated that fish may not be affected by implantation when exceeding the 2% transmitter burden guideline. For example, the swimming performances of rainbow trout (*Oncorhynchus mykiss*) with 6–12% transmitter burdens were not impaired (Brown et al. 2006), nor was the survival of lake sturgeon (*Acipenser fulvescens*) with 4% transmitter burdens (Snobl et al. 2015). With the development of miniaturized acoustic transmitters, researchers may be able to maintain a small transmitter burden while also implanting smaller fish. This advancement in technology further supports a basic assumption in telemetry research; that implanted fish are representative of the overall population of interest (Peven et al. 2005). Understanding the effects of transmitter implantation on small (< 300 mm FL) age-0 juvenile sturgeon is particularly important for researchers investigating early life behavior and survival of wild recruits, and for programs seeking to release and monitor early life stages of hatchery reared sturgeon.

The objective of this laboratory study was to evaluate implantation of a novel, miniaturized acoustic transmitter into age-0 white sturgeon (*Acipenser transmontanus*). Transmitters were implanted in a broad, continuous size distribution of juveniles to determine potential size-dependent effects on transmitter retention, survival, and growth. Two control groups were incorporated into the study design in order to solidify analyses, which ultimately supported recommendations of a minimum size threshold for implantation.

Methods

Sturgeon Acquisition, Implantation, and Rearing

In November 2014, fingerling age-0 white sturgeon (3–30 g) from the Washington Department of Fish and Wildlife Columbia Basin Hatchery (Moses Lake, WA) were obtained and reared in the Aquatic Research Laboratory at the Pacific Northwest National Laboratory (Richland, WA). On 21 January 2015, we graded 360 sturgeon to a continuous size distribution (25–124 g) consisting of 10 weight classes (i.e., 25–34, 35–44, …, 115–124 g), with 36 fish assigned to each weight class. This grading procedure was necessary to evaluate the effects of acoustic transmitter implantation across a wide range of transmitter burdens. We distributed the graded fish into three

subpopulations of 120 individuals (i.e., 12 fish randomly chosen from each weight class), which were held in three separate 600-L circular tanks until the day of tagging. All tanks were supplied with UV-treated and sand-filtered, flow-through Columbia River water. Water flow rates were set at 24 L min^{-1} (~ 2.4 exchanges h^{-1}) and gradually increased to 40 L min^{-1} (~ 4.0 exchanges h^{-1}) to maintain dissolved oxygen concentrations, as tank biomass increased with fish growth. Dissolved oxygen and temperature were maintained at 8.7 ± 0.7 mg L^{-1} and 16.4 ± 0.9 °C (mean ± SD). On a daily basis, we fed sturgeon an ad libitum ration of commercial salmon feed (BioVita Fry, 1.2 mm pellet, Bio-Oregon, Longview, WA), except for 24 h before tagging and examination days. Tagging occurred over three days (one subpopulation per day from 26–28 January 2015).

The study design consisted of three treatments: 1) a group implanted with a non-functioning acoustic transmitter (hereafter referred to as AT), 2) an unmarked group (Control), and 3) a group injected with visible implant elastomer (VIE; Northwest Marine Technologies [NMT], Shaw Island, WA; Table 1). The non-functioning transmitter served as a cost-effective surrogate for the

TABLE 1. General statistics for age-0 white sturgeon on the initial day of implantation (D0), separated by treatment and weight class. The treatments include fish implanted with a non-functioning acoustic transmitter (AT), unmarked (Control), and injected with a visible implant elastomer (VIE). Values represent the mean ± standard deviation with the ranges in parentheses.

Treatment	*n*	Weight class (g)	Fork length (mm)	Weight (g)	Transmitter burden (%)
AT	12	25–34	171.6 ± 6.9 (158–183)	31.4 ± 2.3 (26.5–34.5)	2.2 ± 0.002 (2.1–2.6)
	12	35–44	188.2 ± 5.4 (176–198)	39.6 ± 3.4 (35–44.7)	1.8 ± 0.001 (1.6–2.0)
	12	45–54	200.9 ± 5.1 (192–208)	50.2 ± 3.2 (45.4–54.3)	1.4 ± 0.001 (1.3–1.5)
	12	55–64	216.6 ± 8.1 (202–229)	60.2 ± 3.2 (55.6–64.8)	1.2 ± 0.001 (1.1–1.3)
	12	65–74	223.8 ± 4.5 (217–230)	69.8 ± 2.0 (66.3–73.5)	1.0 ± 0.0003 (1.0–1.1)
	12	75–84	233.4 ± 7.2 (219–247)	79.4 ± 3.0 (74.4–83.7)	0.9 ± 0.0004 (0.8–0.9)
	12	85–94	242.4 ± 5.5 (233–251)	87.9 ± 2.7 (84.2–93.4)	0.8 ± 0.0002 (0.7–0.8)
	12	95–104	250.3 ± 5.4 (240–258)	97.9 ± 2.9 (95.2–104)	0.7 ± 0.0002 (0.67–0.75)
	12	105–114	257.6 ± 6.5 (250–271)	109.9 ± 3.7 (105.9–115.6)	0.6 ± 0.0002 (0.6–0.7)
	12	115–125	270.4 ± 5.4 (259–277)	121.9 ± 2.7 (116.1–125.2)	0.6 ± 0.0001 (0.56–0.60)
Control	12	25–34	170.4 ± 7.5 (158–183)	30.8 ± 3.9 (26.1–39.6)	
	12	35–44	188.3 ± 5.2 (179–196)	40.1 ± 3.9 (35.4–46.3)	
	12	45–54	202.9 ± 4.6 (195–209)	50.6 ± 3.5 (45–56.4)	
	12	55–64	213.4 ± 6.2 (204–227)	59.8 ± 3.3 (56–66)	
	12	65–74	224.6 ± 5.1 (218–234)	70.7 ± 3.4 (65.4–76.6)	
	12	75–84	235.1 ± 5.2 (225–243)	80.0 ± 4.7 (74.4–90.3)	
	12	85–94	247.1 ± 5.4 (238–255)	92.5 ± 2.1 (88.5–95.6)	
	12	95–104	249.6 ± 7.7 (240–262)	100.9 ± 3.4 (95.6–105.8)	
	12	105–114	259.7 ± 7.0 (250–269)	111.3 ± 3.4 (105.7–115.8)	
	12	115–125	266.0 ± 6.7 (249–272)	121.8 ± 3.0 (117.7–126.3)	
VIE	12	25–34	171.2 ± 6.6 (161–185)	30.1 ± 2.3 (26.8–33.7)	
	12	35–44	186.1 ± 7.7 (176–203)	39.1 ± 3.4 (35.2–44.6)	
	12	45–54	200.1 ± 5.3 (190–206)	49.9 ± 2.8 (45.6–53.8)	
	12	55–64	211.8 ± 6.5 (202–226)	59.0 ± 2.5 (55.3–63.8)	
	12	65–74	224.4 ± 5.4 (215–233)	69.5 ± 2.3 (67.2–74.4)	
	12	75–84	233.5 ± 6.0 (227–246)	79.6 ± 2.4 (75.3–82.9)	
	12	85–94	244.8 ± 5.6 (234–254)	90.6 ± 2.3 (85.1–94.2)	
	12	95–104	251.0 ± 10.4 (233–268)	99.4 ± 3.1 (94.9–103.7)	
	12	105–114	258.9 ± 5.7 (251–270)	108.9 ± 3.2 (104–113.7)	
	12	115–125	270.0 ± 5.9 (258–276)	123.0 ± 2.0 (119.5–125.9)	

novel, miniaturized acoustic transmitter because both had the same dimensions (cylindrical, 0.7 g in air, 24.2 mm long, 5.0 mm in diameter), buoyancy, and external housing (Figure 1). The internal acoustic components of the non-functioning AT contained a Passive Integrated Transponder (PIT) tag (12.5 mm long, 2.1 mm in diameter and 0.1 g; Destron Technologies, St. Paul, MN) for cost-effective individual fish ID. The unmarked treatment was a control for tagging effects on fish survival and growth for each subpopulation. The VIE treatment was a control for AT surgery effects on growth of individual fish; VIE injection can be a minimally invasive approach to ID fish as replicates which improves the power and resolution of growth analyses (Brown et al. 2010, Cook et al. 2014).

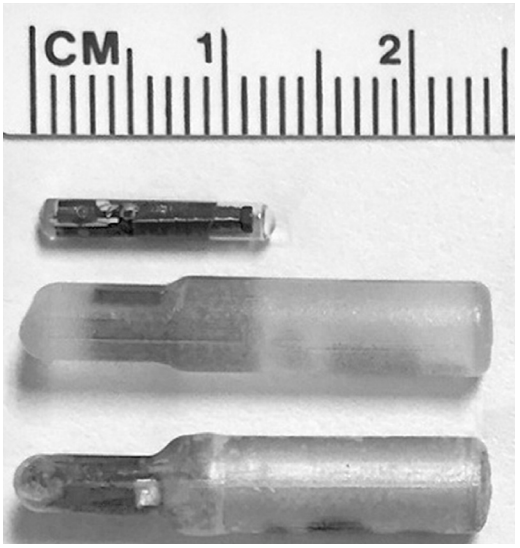

Figure 1. Photo showing the dimensions of a Passive Integrated Transponder tag (top) in relation to the tested non-functioning acoustic transmitter (center) and the functioning acoustic transmitter (bottom).

Surgical Procedures

Immediately prior to surgery, we individually anesthetized fish with buffered 120 mg L^{-1} tricaine methanesulfonate (MS-222) to stage four anesthesia (defined by a complete loss of muscle tone, equilibrium, and spinal reflexes while maintaining a slow but steady opercular rate; Summerfelt and Smith 1990) which took ~ 3.5 min. While anesthetized, each fish was measured for fork length (FL; mm) and weight (g). We randomly assigned 40 individuals (4 sturgeon per weight class) to a treatment group. Non-functional acoustic transmitters were disinfected prior to implantation (submersed in 70% ethanol for at least 20 min, then rinsed with deionized water for at least 10 min and allowed to air dry). Using a sterile #11 scalpel blade, the AT was implanted via a 7–9 mm flank incision on the left side of the sturgeon between the 3rd and 4th ventral scutes (posterior-to-anterior from the pelvic fin) without suture closure. For the VIE treatment, we implemented an individual ID coding scheme consisting of five fluorescent colors (blue, green, pink, red, yellow). Elastomer was injected subcutaneously into four rostrum locations immediately anterior to the barbels (Caroffino et al. 2009) using a handheld 0.3-mL tuberculin syringe (29-gauge needle) coupled with a manual injector (NMT). To minimize implantation bias, one person performed all AT surgeries and another person performed all VIE injections.

After implantation (~ 20–40 s per individual, regardless of AT or VIE), fish were placed in an aerated bucket until they recovered from anesthesia (i.e., achieved equilibrium [≤ 5 min]), and then returned to the 600-L circular rearing tank. Control fish were anesthetized and handled in a similar manner to minimize handling bias, but did not receive a transmitter or VIE injection. Tanks were monitored for mortalities and expelled transmitters daily. In the event of mortality the dead fish was removed, measured for FL and weight, and treatment and date were recorded. All sturgeon were examined on the initial implantation day (D0), and at D7, 14, 21, 28, 56, and 84 post-implantation. On examination days, we anesthetized all fish in buffered 120 mg L^{-1} MS-222, identified treatment as AT, Control, or VIE, and measured for FL and weight.

Fish weights were summed to calculate weekly feeding levels following each examination day. The sturgeon were fed a single daily ration of commercial salmon feed (1.2–1.5 mm pellet) at 1.0% of the mean biomass of the study population (n = 360) for 5 d $week^{-1}$ until D28. Thereafter, we increased feeding to 1.5% of the mean biomass

for 4 d week^{-1} to reduce food competition among growing age-0 conspecifics with a broad size distribution. The experiment concluded on D84 when all study fish were euthanized (250 mg L^{-1} of MS-222).

Statistical Analyses

Evaluations of fish morphometrics after tagging included repeated measures analysis of variance (RMANOVA; mixed model) followed by Tukey's post-hoc tests. Best-fit linear models were selected using Akaike's information criterion (AIC) for comparisons of covariance structure. Potential fixed effects in these models included treatment, examination day (time), D0 covariate metrics (FL and weight) and their interactions; tank or subpopulation effects were accounted for as a random effect. Tested response metrics included specific growth rates as a function of FL and weight (SGR_{FL} and SGR_{W}, respectively; percent growth [mm or g] gained per d [% d^{-1}]; Ricker 1975). Because condition factor (i.e., Fulton's) naturally changes with juvenile fish size, we alternatively tested relative condition factor (K_n; observed weight ÷ calculated weight; Le Cren 1951), in which calculated weight was estimated by linear regression of $\log_{10}$-transformed length-weight data on D0. Transmitter expulsion and fish survival were not analyzed because AT retention was 100% and only one sturgeon (a Control) died over the study period.

A spline regression model was also used in our analyses of fish size thresholds for transmitter implant effects on growth. This model examined the relationship between D0 covariates and specific growth rate for AT and VIE fish. Regression lines predicting SGR for each treatment group diverged at spline points or "knots" within the range D0 covariate values. *F*-tests were used to detect significant differences between spline and common regressions on either side of the knot. Size thresholds were identified at knot locations producing the highest R^2 values that corresponded with significantly different regressions. Type I error (α) was defined at 0.05 for all statistical tests. Model assumptions of homoscedasticity and normality were verified with residual scatter plots and linear trends in quantile-quantile plots. Statistical analyses were performed in R (R Development Core Team 2016) and SAS (Statistical Analysis Software program, Proc Mixed, Version 9.4, SAS Institute, Cary, NC).

Results

Table 1 provides a summary of mean (± SD) FLs, weights, and transmitter burdens (AT only) for each of the treatment groups on the day tagging. Mixed model one-way analysis of variance of D0 morphometrics (FL, weight, and K_n) found no significant differences between fixed effects while controlling for the random effect of tank or subpopulation (i.e., no difference between treatment groups and no interaction of weight class with treatments). During the 84 d study, no transmitters were expelled and only one fish died from an unknown cause (Control fish; recorded on D69). Figure 2 shows mean (± SD) FLs and

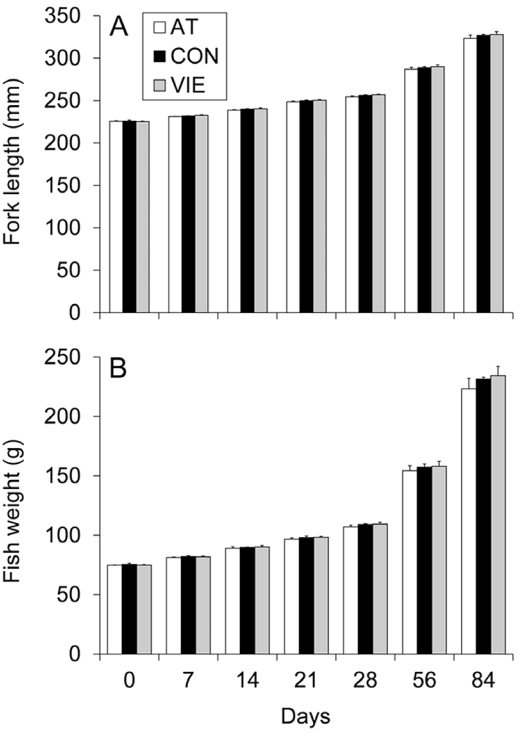

Figure 2. Mean (± SD) FLs and weights (panel A and B respectively) of three treatment groups (AT, Control [CON], VIE) from the initial tagging day (pre-implantation, D0) to the last post-implantation exam (D84).

weights of each treatment group over the study period. Overall, juvenile sturgeon grew rapidly between D0 and D84; average FLs and weights increased respectively by 45% and 206% for all groups. Average condition factors (± SD) for AT, Control, and VIE groups were also similar at the end of the study (0.66 ± 0.01, 0.66 ± 0.01, and 0.67 ± 0.00, respectively).

We analyzed SGR_{FL}, SGR_{W}, and K_n in replicate subpopulations using a best-fit repeated measures model that included treatment, time, and their interaction. Tests of SGR_{FL} found differences between treatments ($F_{(2, 34)} = 4.43$, $P = 0.03$) and exam days ($F_{(5, 34)} = 53.94$, $P < 0.0001$), but no significant interaction of treatments across time ($F_{(10, 34)} = 1.27$, $P = 0.30$); Tukey's post-hoc tests detected differences between AT and VIE ($P = 0.02$), but not between either AT vs. Control ($P = 0.32$) or Control vs. VIE ($P = 0.32$). The effect of time on SGR_{FL} was expected to change as juvenile fish grow—importantly time did not affect SGR_{FL} differently between treatments. Similar tests for SGR_{W} and K_n did not change the overall result of a treatment effect between AT and VIE.

Specific growth rates in AT and VIE groups were further analyzed with replicate individuals using a similar repeated measures model, but also included either a D0 FL or D0 weight covariate for best-fit. The SGR_{FL} of individuals was influenced by treatment effects ($F_{(1, 1422)} = 37.04$, $P < 0.0001$) that interacted with both time ($F_{(5, 1422)} = 7.08$, $P < 0.0001$) and the D0 FL covariate ($F_{(1, 1422)} = 29.85$, $P < 0.0001$). Analysis of SGR_{W} also showed similar treatment ($F_{(1, 1422)} = 36.30$, $P < 0.0001$) and interaction effects with both time ($F_{(5, 1422)} = 2.60$, $P = 0.02$) and the D0 weight covariate ($F_{(1, 1422)} = 20.91$, $P < 0.0001$); conversely, analysis of K_n did not detect any treatment or interaction effects. These results indicate that fish of different size on the day of tagging grew differently between the treatments following implantation. Table 2 provides a summary of SGR_{FL} and SGR_{W} results for AT and VIE groups over the study duration. Tukey's post-hoc tests were performed on time and treatment interaction effects at 10 covariate weights representative of the weight class distribution on D0. Corresponding covariate fork lengths (FL_C) were estimated from linear regression ($\log_{10}[\text{weight}] = 3.0208\log_{10}[FL_C] - 5.2568$, $R^2 = 0.97$) of individual ($n = 359$) length-weight data from D0. For all the tested covariate sizes, AT fish had a lower SGR_{FL} on D7; likewise, SGR_{W} of AT fish was also less, except for the largest implanted fish (> 95 g). Adverse growth effects resulting from AT implantation diminished with time. By D21, only the smaller AT fish (< 207 mm FL and < 55 g) had respectively reduced SGR_{FLs} and SGR_{Ws}—a trend that possibly continued until the end of the study (Table 2).

Spline regression analyses of initial FLs against SGR_{FL} between D0–7 detected a significant threshold for AT implantation left of a knot at 250 mm FL (i.e., for fish ≤ 250 mm FL; Figure 3A). Fish implanted with a transmitter had a reduced SGR_{FL} compared to VIE fish of equivalent size on D0 (Table 3; Figure 3A). Seven AT fish had a negative SGR_{FL} from D0–7 which did not occur in the VIE group. The SGR_{FL} spline break knot shifted to 171 mm FL between D7–14 (Figure 3B); there was also no incidence of negative SGR_{FL} for AT fish. The aforementioned length-weight regression estimated weights (mean ± 95% prediction interval) of 97 ± 13 and 31 ± 4 g that correspond with FL thresholds of 250 and 171 mm; the respective transmitter burdens were 0.7 ± 0.1% and 2.3 ± 0.3% at these weight estimates. Between D14–84, the suggested knot locations were very close to the smallest D0 FLs tested, and the left sides of the splines were not significantly different from a linear regression of the pooled treatments (Table 3). Figure 3B illustrates this pooled regression line which estimates that smaller sturgeon, regardless of treatment, showed significantly (Table 3) higher SGR_{FL} than larger sturgeon (with the exception of AT fish smaller than the determined minimum size threshold [≤ 171 mm FL]).

Results from RMANOVA analyses infer that SGRs were lower in small AT fish throughout the study period (Table 2). Contrastingly, results from spline regression analyses suggest there was growth parity between AT and VIE fish from D21–84 (Table 3). We performed follow-up covariance analyses to determine if equivalently sized fish on D14–56 had different SGRs between AT and VIE in subsequent exam intervals. We found that

TABLE 2. A summary of mixed model repeated measures analyses of specific growth rates respective to fork length (SGR_{FL}) and weight (SGR_W) for two treatment groups (AT, n = 120; VIE, n = 120) of age-0 white sturgeon monitored for 84 days following implantation. Tukey's multiple comparison post-hoc test results are shown for significant (P-values ≤ 0.05 denoted by an *") interaction effects (Time treatment) at 10 covariate weights across a continuous size distribution on study D0. Covariate fork length (FL_C) values were estimated from a D0 length-weight regression equation ($\log_{10}[\text{weight}] = 3.0208\log_{10}[FL_C] - 5.2568$, $R^2 = 0.97$).

Examination Day	Treatment	SGR_{FL} (% d^{-1})	SGR_W (% d^{-1})	D0 covariate values: FL_C (mm)	D0 covariate values: Weight (g)	SGR_{FL} Time treatment P-values	SGR_W Time treatment P-values
7	AT	0.35 ± 0.18	1.11 ± 0.64	160	25	< 0.0001*	< 0.0001*
	VIE	0.46 ± 0.14	1.36 ± 0.46	178	35	< 0.0001*	< 0.0001*
				194	45	< 0.0001*	< 0.0001*
				207	55	< 0.0001*	< 0.0001*
				219	65	< 0.0001*	< 0.0001*
				230	75	< 0.0001*	< 0.001*
				239	85	< 0.0001*	< 0.01*
				248	95	< 0.0001*	0.04*
				257	105	< 0.001*	0.26
				264	115	< 0.01*	0.74
14	AT	0.45 ± 0.14	1.31 ± 0.49	160	25	< 0.0001*	< 0.001*
	VIE	0.48 ± 0.14	1.44 ± 0.43	178	35	< 0.001*	< 0.001*
				194	45	< 0.01*	< 0.01*
				207	55	0.07	0.02*
21	AT	0.58 ± 0.14	1.19 ± 0.41	160	25	< 0.001*	< 0.01*
	VIE	0.60 ± 0.15	1.29 ± 0.37	178	35	< 0.01*	< 0.01*
				194	45	0.06	0.02*
28	AT	0.35 ± 0.12	1.50 ± 0.42	160	25	< 0.01*	0.04*
	VIE	0.36 ± 0.12	1.55 ± 0.42				
56	AT	0.43 ± 0.09	1.31 ± 0.29	160	25	< 0.01*	0.21
	VIE	0.44 ± 0.10	1.32 ± 0.34	178	35	0.05*	0.45
84	AT	0.43 ± 0.08	1.32 ± 0.31	160	25	< 0.01*	< 0.01*
	VIE	0.44 ± 0.09	1.41 ± 0.33	178	35	0.02*	0.01*
				194	45	0.18	0.05*

TABLE 3. A summary of R^2-values, P-values, and spline knot locations predicting D0 FL effects on SGR_{FL} between AT and VIE treatments on different examination days post-implantation. A P-value ≤ 0.05 (denoted by an "*") on the left side of the spline knot (i.e., the point at which a size threshold is observed) indicates the spline regression significantly improves model fit compared to a linear regression. Thresholds for D0 fish length effects on SGR_{FL} of the AT treatment were 250 mm FL (D7) and 171 mm FL (D14).

Examination day	Linear regression: R^2	Linear regression: P (> F)	Spline regression: Knot location R^2	Spline regression: Knot location (mm FL)	Spline regression: Left side	Spline regression: Right side
7	0.003	0.41	0.26	250	$P\ (F_{(2, 170)} > 34.2) < 0.0001$*	$P\ (F_{(2, 59)} > 0.11) = 0.89$
14	0.07	< 0.0001	0.19	171	$P\ (F_{(2, 9)} > 8.9) = 0.01$*	$P\ (F_{(2, 221)} > 1.00) = 0.37$
21	0.14	< 0.0001	0.18	170	$P\ (F_{(2, 5)} > 3.5) = 0.11$	$P\ (F_{(2, 222)} > 2.35) = 0.10$
28	0.11	< 0.0001	0.14	170	$P\ (F_{(2, 5)} > 4.8) = 0.07$	$P\ (F_{(2, 222)} > 1.15) = 0.32$
56	0.15	< 0.0001	0.20	170	$P\ (F_{(2, 5)} > 4.2) = 0.08$	$P\ (F_{(2, 223)} > 0.01) = 0.99$
84	0.19	< 0.0001	0.23	169	$P\ (F_{(2, 4)} > 2.8) = 0.17$	$P\ (F_{(2, 228)} > 0.57) = 0.57$

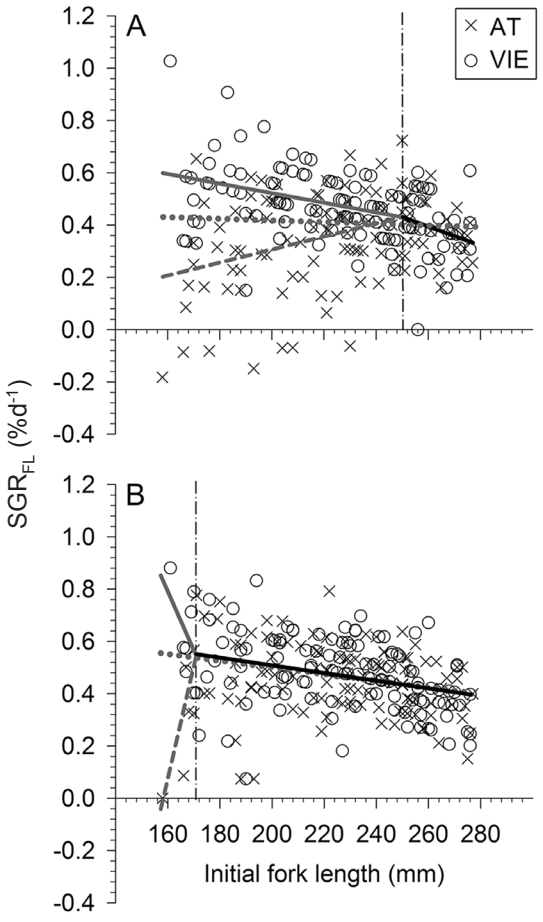

Figure 3. Spline regression analyses of initial (D0) fork length (FL) and specific growth rates (SGR_{FL}) of age-0 white sturgeon either implanted with a non-functioning acoustic transmitter (AT; n = 120) or injected with visible implant elastomer (VIE; n = 120). Minimum D0 size thresholds (vertical dashed and dotted line) for implantation were identified by a spline break (i.e., knot) for SGR_{FL} recorded on Days 7 and14 post-implantation (panels A and B respectively). Thresholds were detected at 250 mm for D7 ($P < 0.0001$) and 171 mm FL for D14 ($P = 0.01$). Significantly different regression lines left of the knot predict SGR_{FL} for the AT (dashed) and VIE (solid) groups. Linear regression lines of the pooled treatments are also shown (dotted).

SGR_W was not significantly affected by treatment or covariate interaction effects between D21–84; however, SGR_{FL} was influenced by treatment ($F_{(1, 233)} = 10.10$, $P < 0.01$) and interaction effects ($F_{(1, 233)} = 9.21$, $P < 0.01$) on D21, but not thereafter. Tukey's post-hoc tests determined that AT fish < 239 mm FL on D14 had reduced SGR_{FLs} from D14–21 compared to VIE fish of equivalent size. These results suggest that the AT test population regained their growth potential between D28–84, albeit seven days later than was inferred from spline regression analyses.

Discussion

This investigation is the first to evaluate transmitter retention, survival, and growth of very small (158–277 mm FL) age-0 white sturgeon implanted with a surrogate, non-functioning acoustic transmitter that mimicked a novel, miniaturized (0.7 g), long battery life (up to 365 d) acoustic transmitter. We found no evidence of implantation effects on transmitter retention or fish survival for sturgeon as small as 158 mm FL (26 g; 2.6% transmitter burden). Transmitter implantation caused minor adverse effects on fish growth over an 84-d evaluation period after tagging. Therefore, we recommend testing of this new miniaturized acoustic transmitter in field studies, which may aid researchers and managers of supplementation programs seeking to monitor and track age-0 juvenile sturgeon.

Overall comparisons of fish growth after tagging suggest that fish implanted with either AT or VIE grew similarly to unmarked control fish (Figure 2); repeated measures analysis of specific growth rates and relative condition factor in these groups confirmed this result. Further analyses of individual fish in VIE and AT groups detected reduced SGRs in smaller AT fish throughout the study period (Table 2). However, spline regression analyses did not detect differences in SGRs between AT and VIE fish from D21–84 (Table 3). To better interpret these results, we also performed exam-interval covariate analyses and found that the AT test population regained their growth potential seven days later than was inferred from spline regression analyses. Ultimately, we concluded that a minimum size threshold of < 171 mm FL was appropriate for AT implantation—concomitant with an expectation that significant adverse effects on growth may occur up to 21 days post-implantation, but only very minor effects thereafter.

The recorded FLs and weights of AT fish were slightly less than those of VIE fish on each

post-implantation exam day (Figure 2). Our repeated measures analyses of SGRs captured the significance of this treatment interaction with time. Interestingly, average SGRs of smaller AT fish did not exceed those of larger VIE fish at the end of the study (Table 2)—a result that conflicts with normal growth patterns in juvenile fish (i.e., intraspecies SGRs are typically greater in smaller fish; Ricker 1975). Our results from repeated measures of SGRs might reflect intracohort food competition that continually favored higher SGRs in VIE fish, despite their larger size. It is also possible that transmitter implants adversely affected growth of smaller fish (< 207 mm FL or < 55 g on D0) into the D28–48 study period, but the effects were too minor for detection in our spline regression and exam-interval covariate analyses.

It is also interesting that FL was generally a more sensitive growth metric than weight in all of our analyses, especially in smaller fish. This was evidenced by greater incidence of highly significant *P*-values in repeated measures analyses of FL (Table 2) and enhanced detection of effects in exam-interval covariate analyses. Consequently, we only reported on FL in spline regression analyses to further elucidate a size threshold for AT implantation. In general, fish length is more commonly recorded by fish biologists, hatchery staff, and tagging crews who are measuring individual juvenile sturgeon in the field (i.e., recording individual weights of small fish with a digital scale can be labor intensive and prone to measurement errors). We observed that even in a laboratory setting, weights of smaller fish may be stochastically confounded by water adhesion to the skin and presence in both the buccal and opercular cavities. Weights of smaller fish are certainly informative to assessments of condition factor, but we did not detect any significant differences in K_n between treatments over the study duration.

The initial reduced growth rate documented in the present study is comparable to results from other implantation studies. For example, Crossman et al. (2013) noted a significant difference in specific growth rates among treatment groups of shortnose sturgeon (*Acipenser brevirostrum*; 35.7 cm mean FL, 318.1 g mean weight, 1.5% mean transmitter burden), but the effect decreased (i.e., became nonsignificant) over time. Adams et al. (1998) also found that surgically-implanted juvenile Chinook salmon (*Oncorhynchus tshawytscha*; 135 mm mean FL, 28 g mean weight, 3.6% mean transmitter burden) had a significantly lower growth rate in the first 21 d compared to controls, but this difference disappeared by 54 d post-implantation.

The release of age-0 hatchery sturgeon into rivers or lakes can result in low survival and slow growth during their first year living in the wild (Ireland et al. 2002, Justice et al. 2009, Steffensen et al. 2010, McDougall et al. 2014b). While other investigators have evaluated transmitter retention, survival, and growth in sturgeon (Boone et al. 2013, Crossman et al. 2013, Miller et al. 2014), the present study is unique because the sturgeon were the smallest that have been implanted with an acoustic transmitter while maintaining a relatively low transmitter burden (158 mm FL; 26 g; 2.6 % maximum transmitter burden). This was possible because of the development of a new, miniaturized acoustic transmitter. These findings, along with the relatively long battery life, may advance our understanding of movement patterns, habitat preferences, and assessments of year-class strength and natural recruitment of small age-0 sturgeon in field studies. Currently, the smallest published size of sturgeon implanted with an acoustic transmitter that has been used in a field study is reported to be 90 ± 10 g (Snobl et al. 2015), which is substantially larger than the smallest fish (26 g) implanted in the present study. Other studies have reported that the estimated survival rates of age-0 or age-1 juvenile white sturgeon (i.e., ≤ 250 mm FL) are typically lower for smaller fish than for larger fish (Justice et al. 2009, Steffensen et al. 2010). Reducing the implantable fish size to 158 mm FL (26 g) is important because it affects the ability to monitor juvenile sturgeon using acoustic telemetry during their first winter—a period which may be critical to survival, recruitment, and fisheries productivity.

Recommendations for a minimum fish size for transmitter implantation will depend upon specific objectives of the investigator(s). Our study results

support implanting sturgeon as small as 158 mm FL. However, future research with this transmitter should consider evaluations of transmitter effects on swimming behavior and susceptibility to predation. For acoustic telemetry studies involving hatchery sturgeon, we recommend implanting age-0 sturgeon > 171 mm FL (~ 31 g; 2.3% transmitter burden), because hatcheries will often hold implanted fish for several weeks post-implantation for inspections of transmitter loss and fish health prior to release. Under those circumstances, transmitter effect concerns may include short-term (21 d) decreases in feeding behavior and growth. For studies where fish growth is a major concern, or for instances where hatchery fish are released immediately after implantation, we recommend implanting sturgeon > 250 mm FL (~ 97 g; 0.7% transmitter burden).

Before conducting a large project, field testing with these small fish should be conducted to provide insight and proof of concept for researchers who plan to implant fish < 250 mm. This conservative recommendation for implantable fish size still remains considerably smaller than the initial lengths of sturgeon implanted with acoustic transmitters in previous laboratory research (57.4 cm mean FL [Siberian sturgeon; *Acipenser baeri*; Boone et al. 2013], 35.7 cm mean FL [shortnose sturgeon] and 37.0 cm mean FL [Atlantic sturgeon; *Acipenser oxyrinchus*; Crossman et al. 2013], and 45.4 cm mean total length [green sturgeon; *Acipenser medirostris*; Miller et al. 2014]). These recommendations are designed to minimize adverse transmitter effects caused by implantation and to give researchers a tool to address questions regarding age-0 sturgeon early life ecology. Using a miniaturized transmitter could reduce bias in telemetry studies and benefit investigations with transmitter effect concerns (Johnson et al. 2014, Miller et al. 2014). It may also advance research of year class strength, recruitment, and survival (Bates 2013) for wild-caught and hatchery fish through monitoring of small fish.

Acknowledgments

We thank Manitoba Hydro, Idaho Power Company, and the Laboratory Directed Research and Development Program at the Pacific Northwest National Laboratory (PNNL) for funding support. We would also like to thank the Washington Department of Fish and Wildlife Columbia Basin Hatchery for providing us with sturgeon and PNNL staff who were instrumental in this study: Z. Daniel Deng and Huidong Li for acoustic transmitter development, Tim Linley for his assistance in fish husbandry and laboratory use, and Jill Janak, Sadie Montgomery, Jose Vasquez, Ethan Green, and Erika Cutsforth for their assistance with implantation.

Literature Cited

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.

Barth, C. B., W. G. Anderson, L. M. Henderson, and S. J. Peake. 2011. Home range size and seasonal movement of juvenile lake sturgeon in a large river in the Hudson Bay Drainage Basin. Transactions of the American Fisheries Society 140:1629-1641.

Bates, P. 2013. Report A. Snake River white sturgeon conservation plan—Implementation of Measures for White Sturgeon in the Shoshone Falls to C. J. Strike and Hells Canyon Reaches of the Snake River. 2011–2012 Annual Report to Idaho Power Company, Boise, ID.

Bates, P., J. Chandler, K. Lepla, and K. Steinhorst. 2014. Using mark-recapture data in an individual-based model to evaluate length-at-age differences between two Snake River white sturgeon (*Acipenser transmontanus* Richardson, 1836) populations in Idaho, USA. Journal of Applied Ichthyology 30:1319-1327.

Boone, S. S., S. J. Divers, A. C. Camus, D. L. Peterson, C. A. Jennings, J. L. Shelton, and S. M. Hernandez. 2013. Pathologic and physiologic effects associated with long-term intracoelomic transmitters in captive Siberian sturgeon. North American Journal of Fisheries Management 33:869-877.

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, and K. A. Deters. 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.

Caroffino, D. C., T. M. Sutton, and M. S. Lindberg. 2009. Abundance and movement patterns of age-0 juvenile lake sturgeon in the Peshtigo River, Wisconsin. Environmental Biology of Fishes 86:411-422.

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 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.

Crossman, J. A., K. L. Hammell, and M. K. Litvak. 2013. Experimental examination of surgical procedures for implanting sonic transmitters in juvenile shortnose sturgeon and Atlantic sturgeon. North American Journal of Fisheries Management 33:549-556.

Hondorp, D. W., C. M. Holbrook, and C. C. Krueger. 2015. Effects of acoustic tag implantation on lake sturgeon *Acipenser fulvescens*: lack of evidence for changes in behavior. Animal Biotelemetry 3:1-13.

Ireland, S. C., R. C. P. Beamesderfer, V. L. Paragamian, V. D. Wakkinen, and J. T. Siple. 2002. Success of hatchery-reared juvenile white sturgeon (*Acipenser transmontanus*) following release in the Kootenai River, Idaho, USA. Journal of Applied Ichthyology 18:642-650.

Johnson, J. L., W. D. Hintz, J. E. Garvey, Q. E. Phelps, and S. J. Tripp. 2014: Evaluating growth, survival and swimming performance to determine the feasibility of telemetry for age-0 pallid sturgeon (*Scaphirhynchus albus*). The American Midland Naturalist Journal 171:68-77.

Justice, C., B. J. Pyper, R. C. P. Beamesderfer, V. L. Paragamian, P. J. Rust, M. D. Neufeld, and S. C. Ireland. 2009. Evidence of density- and size-dependent mortality in hatchery-reared juvenile white sturgeon (*Acipenser transmontanus*) in the Kootenai River. Canadian Journal of Fisheries and Aquatic Sciences 66:802-815.

Lacroix, G. L., D. Knox, and P. McCurdy. 2004. Effects of implanted dummy acoustic transmitters on juvenile Atlantic salmon. Transactions of the American Fisheries Society 133:211-220.

Le Cren, E. D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). Journal of Animal Ecology 20:201-219.

Lu, J., Z. D. Deng, H. Li, M. J. Myjak, J. J. Martinez, J. Xiao, R. S. Brown, and S. S. Cartmell. 2016. A small long-life acoustic transmitter for studying the behavior of aquatic animals. Review of Scientific Instruments 87:114902.

McDougall, C. M., C. C. Barth, J. K. Aiken, L. M. Henderson, M. A. Blanchard, K. M. Ambrose, C. L. Hrenchuk, M. A. Gillespie, and P. A. Nelson. 2014a. How to sample juvenile lake sturgeon (*Acipenser fulvenscens* Rafinesque, 1817), in Boreal Shield rivers using gill nets, with an emphasis on assessing recruitment patterns. Journal of Applied Ichthyology 30:1402-1415.

McDougall, C. M., D. J. Pisiak, C. C. Barth, M. A. Blanchard, D. S. MacDonell, and D. Macdonald. 2014b. Relative recruitment successs of stocked age-1 vs age-0 lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817) in the Nelson River, northern Canada. Journal of Applied Ichthyology 30:1451-1460.

McMichael, G. A., M. B. Eppard, T. J. Carlson, J. A. Carter, B. D. Ebberts, R. S. Brown, M. Weiland, G. R. Ploskey, R. A. Harnish, and Z. D. Deng. 2010. The juvenile salmon acoustic telemetry system: A new tool. Fisheries 35:9-22.

Miller, E. A., H. E. Froehlich, D. E. Cocherell, M. J. Thomas, J. J. Cech Jr., P. Klimley, and N. A. Fangue. 2014. Effects of acoustic tagging on juvenile green sturgeon incision healing, swimming performance, and growth. Environmental Biology of Fishes 97:647-658.

Nelson, T. C., P. Doukakis, S. T. Lindley, A. D. Schreier, J. E. Hightower, L. R. Hildebrand, R. E. Whitlock, and M. A. H. Webb. 2014. Research tools to investigate movements, migrations, and life history of sturgeons (Acipenseridae), with an emphasis on marine-oriented populations. Plos One 8:1-22.

Neufeld, M. D., and P. J. Rust. 2009. Using passive sonic telemetry methods to evaluate dispersal and subsequent movements of hatchery-reared white sturgeon in the Kootenay River. Journal of Applied Ichthyology 25:27-33.

Parsley, M. J., N. D. Popoff, B. K. Van Der Leeuw, and C. D. Wright. 2008. Seasonal and diel movements of white sturgeon in the Lower Columbia River. Transactions of the American Fisheries Society 137:1007-1017.

Peterson, D. L., P. Vecsei, and C. A. Jennings. 2007. Ecology and biology of the lake sturgeon: a synthesis of current knowledge of a threatened North American *Acipenseridae*. Reviews in Fish Biology and Fisheries 17:59-76.

Peven, C., A. Giorgi, J. Skalski, A. Grassell, S. G. Smith, T. Counihan, R. Perry, and S. Bickford. 2005. Guidelines and recommended protocols for conducting, analyzing, and reporting juvenile salmonid survival studies in the Columbia River basin. Report to the U.S. Army Corps of Engineers, Portland, OR.

R Development Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at http://www.R-project.org (accessed September–November 2016).

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Fisheries

Research Board of Canada Bulletin 191. Fisheries Research Board, Ottawa.

Skalski, J. R., M. B. Eppard, G. R. Ploskey, M. A. Weiland, T. J. Carlson, and R. L. Townsend. 2014. Assessment of subyearling Chinook salmon survival through the federal hydropower projects in the main-stem Columbia River. North American Journal of Fisheries Management 34:741-752.

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. Chelan County Public Utility District, Wenatchee, WA.

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

Steffensen, K. D., L. A. Powell, and J. D. Koch. 2010. Assessment of hatchery-reared pallid sturgeon survival in the lower Missouri River. North American Journal of Fisheries Management 30:671-678.

Summerfelt, R. C. and L. S. Smith. 1990. *In* C. B. Schreck and P. B. Moyle (editors). Anesthesia, Surgery, and Related Techniques. Methods for Fish Biology. American Fisheries Society, Bethesda, MD. Pp. 213-272.

Trested, D. G., K. Ware, R. Bakal, and J. J. Isley. 2011. Microhabitat use and seasonal movements of hatchery-reared and wild shortnose sturgoen in the Savannah River, South Carolina–Georgia. Journal of Applied Ichthyology 27:454-461.

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

Winter, J. D. 1983. Underwater biotelemetry. *In* L. A. Nielson and D. L. Johnson (editors). Fisheries Techniques. American Fisheries Society, Bethesda, MD. Pp. 371-395.

Zale, A. V., C. Brooke, and W. C. Fraser. 2005. Effects of surgically implanted transmitter weights on growth and swimming stamina of small adult westslope cutthroat trout. Transactions of the American Fisheries Society 134:653-660.

Received 01 July 2016
Accepted 29 November 2016