North American Journal of Fisheries Management
© 2018 American Fisheries Society
ISSN: 0275-5947 print / 1548-8675 online
DOI: 10.1002/nafm.10198

ARTICLE

Juvenile Chinook Salmon Survival When Exposed to Simulated Dam Passage After Being Implanted With a New Microacoustic Transmitter

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Abstract
The current minimum size for tagging Chinook Salmon *Oncorhynchus tshawytscha* in the Columbia River basin with acoustic transmitters is ≥95 mm FL. Using a newly developed cylindrical microacoustic transmitter (AT; weight in air, 0.22 g), our objective was to evaluate the minimum size of Chinook Salmon for tagging. We measured Chinook Salmon survival and the retention of transmitters and viscera after their exposure to rapid decompression (*n* = 399) or shear forces (*n* = 308) that simulated dam passage. Fish (69–107 mm FL) were implanted with an AT (AT-only) or an AT and a PIT tag (weight in air = 0.10 g; AT+PIT) through a 3-mm incision with no sutures, or did not receive an incision or tag (untagged control fish). Tag burden averaged 2.9% (range, 1.4–6.2%) in the AT-only group and 4.2% (range, 2.0–7.9%) in the AT+PIT group. Proportional survival and the retention of transmitters and viscera was significantly lower for AT-only (0.70) and AT+PIT (0.54) fish than for untagged fish (0.85) after their exposure to pressure change scenarios. No transmitters were fully expelled, but 9% of AT-only and 22% of AT+PIT salmon had protruding viscera or transmitters. Following shear exposure, the proportional survival and retention of transmitters and viscera was significantly lower for AT-only (0.70) and AT+PIT (0.61) fish than for untagged fish (0.98). Visceral expulsion was attributed to 90% and 93% of mortal injuries in AT-only and AT+PIT fish, respectively. In both tests the tagged fish suffered more mortal injuries and death than did untagged fish over the range of tag burdens tested, and no tag burden threshold below which tagged and untagged fish performed similarly was found. As such, a generalized linear model that included tag burden as a predictor variable provided the best fit to the survival data. Without a significant tag burden threshold, we recommend the minimum size for tagging Chinook Salmon using the transmitters and PIT tags evaluated, applied with a 3-mm incision and no sutures, should remain at 95 mm FL.

Acoustic telemetry is routinely used around the world to monitor fisheries populations and their interaction with the environment (Mitamura et al. 2008; Kawabata et al. 2010; Cooke et al. 2011; Dudgeon et al. 2015). One widely used acoustic telemetry tool is the juvenile salmon acoustic telemetry system (JSATS) (McMichael et al. 2010) that was originally developed to measure survival rates of juvenile Pacific salmon *Oncorhynchus* spp. and steelhead *O. mykiss* (anadromous Rainbow Trout) that make seaward migrations through the federal Columbia River power system (FCRPS, Pacific Northwest). Currently 13 salmon and steelhead populations inhabiting the FCRPS, including some stocks of Chinook Salmon *O. tshawytscha,* are listed as threatened or endangered under the U.S. Endangered Species Act of 1973 (ESA 1973). Performance standards in the 2008 Biological Opinion (BiOp) prepared for the FCRPS (NOAA 2008) set minimum downstream dam passage survival rates for smolts (93–96%) and maximum SEs of the survival estimates (≤1.5%). Tagging juvenile salmon with JSATS technology

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Received November 17, 2017; accepted June 3, 2018
Published online xxx xx, 2018

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	N A F M	10198	WILEY	Dispatch: 20.6.18	CE:
	Journal Code	Manuscript No.		No. of pages: 13	PE:

continues to be the primary tool used in the BiOp performance standard studies to measure juvenile salmon survival rates within the FCRPS (Weiland et al. 2009; Harnish et al. 2012; Skalski et al. 2014).

To have confidence in studies that use JSATS, it is important to know that investigators used the transmitters that minimally affected the fish's survival and performance. If the performance of tagged fish is negatively affected, the survival estimates from performance standard studies may be biased or the error associated with those estimates will be too high and study results will not accurately or precisely reflect the true survival rate of the population. To reduce the potential error in survival rates caused from the negative effects of the transmitter on fish performance and survival, researchers in the Columbia River basin recommend only tagging juvenile salmon and steelhead that are at least 95 mm FL (McMichael et al. 2011; USACE 2011). However, this minimum size restriction may limit the interpretation of study findings. It would be beneficial to tag smaller fish to ensure that the entire size range of the population is studied and represented in management decisions. To that end, a novel, cylindrical, microacoustic JSATS transmitter (diameter, 3.3 mm; length, 15 mm; weight in air, 0.22 g) was developed, weighing about 30% less in air than the previous JSATS transmitter model (Chen et al. 2014; Deng et al. 2015). In a field study of juvenile Chinook Salmon in the Snake River, survival rates were significantly higher in the fish tagged with the new microacoustic transmitter than in fish tagged with the previous JSATS transmitter; however, the minimum size of fish tagged was 95 mm FL (Deng et al. 2017). Those authors recommended additional evaluations of the minimum size of juvenile salmonids that could be implanted with the new microacoustic transmitter without affecting fish performance.

Laboratory studies on juvenile salmon tagged with the new microacoustic transmitter used in this study have examined swimming performance and predator avoidance (Walker et al. 2016) as well as survival, transmitter expulsion, growth, and wound healing (Liss et al. 2016). The swimming performance and predator avoidance experiments (Walker et al. 2016) determined that predator avoidance was not affected by the presence of the microacoustic transmitter. However, juvenile Chinook Salmon tagged with the transmitter had lower swimming performance than untagged fish among individuals < 79 mm FL. Based on the survival study, Liss et al. (2016) suggested that placement of either a microacoustic transmitter or a microacoustic transmitter and a PIT tag had greater effects on smaller juvenile fall Chinook Salmon than on larger fish. Although a specific size threshold was not determined, mortality and tag expulsion were less likely among fish > 90 mm FL. While these laboratory studies suggested the new microacoustic transmitter would potentially be useful in tagging fish < 95 mm FL, the effects of rapidly decreasing pressures (i.e., rapid decompression) or shear forces associated with passage through the FCRPS have not been evaluated.

When juvenile salmon pass through hydropower turbines like those found in the FCRPS, they are exposed to rapid decompression. Decompression causes the gas within a fish's swim bladder to expand in proportion to the reduction in pressure; if this occurs rapidly, the fish is not able to regulate the amount of gas in its swim bladder or circulatory system (Stephenson et al. 2010; Brown et al. 2012). This rapid expansion of gas within fish may lead to mortality or a suite of injuries (i.e., barotraumas), such as exophthalmia, swim bladder rupture, embolism, and hemorrhaging, or possibly result in the expulsion of the transmitter or internal organs (Stephenson et al. 2010; Brown et al. 2014; Pracheil et al. 2015). Brown et al. (2012) determined that the main factor associated with mortal injury of juvenile fish exposed to pressure changes that simulate passage through hydropower turbines is the ratio between acclimation pressure and the lowest exposure pressure (referred to as the nadir pressure); the natural-log transformation of this ratio is referred to as the log ratio pressure change. Carlson et al. (2012) showed that the log ratio pressure change and tag burden (i.e., weight of the transmitter relative to the weight of the fish in air) were the best predictors of mortal injury in juvenile Chinook Salmon exposed to pressure treatments and may bias survival estimates of fish passing through turbines in the FCRPS by as much as 20%. Single-suture incision closures retained larger versions of the JSATS transmitters (diameter, 3.8 mm; length, 12 mm; width, 5.2 mm; weight in air, 0.38 g) as well as two-suture incision closures did in juvenile salmonids (95–135 mm FL; mean tag burden, 2.6%) exposed to rapid decompression; however, expulsion of viscera was higher in fish with one suture than in those with two (Boyd et al. 2011). Even though the use of a single suture was not recommended by Boyd et al. (2011) for closing 6-mm incisions, the smaller size of the new microacoustic transmitter allows the use of a smaller incision (3 mm), which may enable researchers to successfully forgo the use of sutures altogether. However, this assumption needs to be evaluated.

Passage through hydropower dams can also expose fish to shear forces, which are created when two masses of water moving in different directions intersect with each other or when water slows and then speeds up as it contacts solid structures such as wicket gates, turbine runners, and turbine blades (Čada et al. 2007). Spillway passage over dams also creates shear environments when fish are entrained in fast-moving water as they enter the turbulent shear flow zone in the transition between the spillway chute and the tailrace (Deng et al. 2010). The effects of shear on fishes, especially salmonids, has been studied

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(Neitzel et al. 2000, 2004; Johnson et al. 2003; Deng et al. 2005, 2010), and injuries associated with shear forces may include bruising, descaling, loss of equilibrium, disorientation, increased susceptibility to predation, or damage to viscera (Deng et al. 2005; Pracheil et al. 2015; Colotelo et al. 2016). However, studies exposing juvenile salmon to shear forces after being implanted with an internal transmitter have not been done.

The negative effects caused by sudden pressure changes or exposure to extreme shear forces, which could occur at the same time at some facilities, may be influenced by an increasing tag burden or by the method of transmitter implantation (i.e., a transmitter implanted into the body cavity through an incision with no sutures may be more likely to be expelled than a traditional implantation using a sutured incision). An examination of the expulsion of transmitters and viscera across a range of fish sizes and tag burdens is needed to provide important information for determining a minimum size criterion for implanting salmonids with the new microacoustic transmitter using an incision with no sutures, especially for studies being conducted to address mandated survival metrics in the FCRPS BiOp. The objective of the current study was to identify a minimum size threshold for tagging juvenile Chinook Salmon with the new microacoustic transmitter. To achieve this, we examined survival and the retention of transmitters and viscera after the exposure of juvenile Chinook Salmon to rapid decompression or shear forces that are representative of conditions experienced by juvenile salmon as they pass through the FCRPS through either hydroturbines, dam bypasses, or spillways.

METHODS

Source of fish, transmitter, and study groups.— Study fish were juvenile spring Chinook Salmon from Leavenworth National Fish Hatchery (U.S. Fish and Wildlife Service) raised from eggs at the Aquatic Research Laboratory at the Pacific Northwest National Laboratory (PNNL), Washington. Fish were randomly sampled and assigned to one of four size-classes (Figure 1). We stratified sampling by size to ensure adequate samples were collected that represented the lower end of the range of lengths (i.e., small fish) of juvenile Chinook Salmon collected at hydropower facilities in the FCRPS over the past 10 years (source of data: www.fpc.org) and also to ensure fish were sampled that were at least 10 mm larger than the current recommended minimum size for tagging juvenile salmon with the JSATS transmitter (i.e., ≥95 mm FL). The study fish ranged from 69 to 107 mm FL and from 3.5 to 16.2 g (Table 1).

For this study, we used nonfunctioning microacoustic transmitters manufactured at PNNL that were similar in size, weight, and other dimensions as the functional microacoustic transmitters: mean length, 15.0 mm; mean diameter, 3.3 mm; mean weight in air, 0.22 g; and mean weight in water, 0.11 g. The microacoustic transmitters used in this study were small enough to be injected into the coelom using an 8-gauge needle (Cook et al. 2014; Liss et al. 2016). However, Cook et al. (2014) found that juvenile Chinook Salmon (66–108 mm FL) with an unsutured incision had the smallest wound area and faster wound healing time than did fish with injected tags. For dynamic environments, such as those associated with severe changes in pressure and shear, Cook et al. (2014) recommended an incision method, and we opted to implant the tags using an unsutured incision.

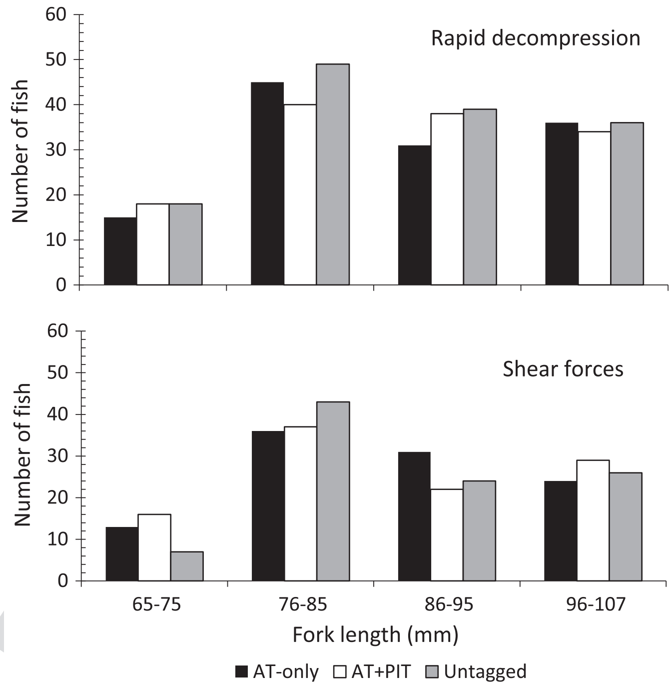

FIGURE 1. Juvenile Chinook Salmon were stratified and sampled by FL and assigned to four size-classes within each of three treatment groups: acoustic tag only (AT-only), acoustic tag and PIT tag (AT+PIT), and untagged fish. The top panel shows fish tested in the rapid decompression tests, while the bottom panel shows fish exposed to shear forces.

Three treatment groups were studied: microacoustic transmitter only (AT-only), AT and a PIT tag (AT+PIT), and untagged control (no incision or tag). A treatment group that included PIT tags (i.e., AT+PIT) was incorporated into the study because juvenile survival studies in the FCRPS normally combine a PIT tag with an acoustic transmitter to identify fish that enter barge transportation or juvenile sampling facilities during their downstream migration (Skalski et al. 2014). The PIT tags (Destron Technologies, St. Paul, Minnesota) measured 15.1 mm in length and 3.5 mm in diameter, and weighed 0.10 g in air. Tag burden averaged 2.9% (range, 1.4–6.2%) in the AT-

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TABLE 1. Chinook Salmon were tagged or handled in a similar manner and then exposed to rapid decompression or shear forces that simulated hydroturbine passage. Treatments were acoustic transmitter (AT-only), acoustic transmitter and a PIT tag (AT+PIT), and untagged controls. The mean and SD of FL, weight (*W*), and transmitter burden (transmitter weight as a percentage of body weight) are provided for each treatment.

		FL (mm)		*W* (g)		Transmitter burden (%)	
Treatment	*n*	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Rapid decompression							
AT-only	127	87.7 ± 10.0	70–105	8.5 ± 3.1	3.5–15.8	2.9 ± 1.0	1.4–6.2
AT+PIT	130	87.5 ± 9.9	70–105	8.5 ± 3.0	4.0–16.2	4.2 ± 1.5	2.0–7.9
Untagged	142	87.5 ± 9.9	69–107	8.4 ± 3.1	3.5–15.8		
Total	399	87.6 ± 9.9	69–107	8.4 ± 3.0	3.5–16.2		
Shear forces							
AT-only	104	87.2 ± 9.7	70–104	8.5 ± 2.9	3.9–15.6	2.9 ± 1.0	1.4–5.5
AT+PIT	104	87.0 ± 9.9	71–105	8.4 ± 2.9	4.2–14.9	4.2 ± 1.4	2.1–7.5
Untagged	100	87.3 ± 9.4	72–106	8.5 ± 2.8	4.1–15.1		
Total	308	87.2 ± 9.6	70–106	8.5 ± 2.9	3.9–15.6		

only group and 4.2% (range, 2.0–7.9%) in the AT+PIT group (Table 1). The tag burdens were higher in the AT+PIT group owing to the additional weight of the PIT tag (0.1 g), but tag burdens for AT-only and AT+PIT fish were similarly distributed within rapid decompression and shear force experiments (Figure 2). There was a strong relationship between FL and tag burden (*B*) in each tagging group: AT-only: $B = 59.26^{(-0.035 \times \text{FL})}$, $R^2 = 0.96$ and AT+PIT: $B = 83.70^{(-0.035 \times \text{FL})}$, $R^2 = 0.95$.

Exposure to rapid decompression.— Rapid decompression tests were performed using the mobile aquatic barotrauma laboratory located at PNNL (Stephenson et al. 2010). From early October to mid-December 2016, a total of 399 fish in the size range of 69 to 107 mm FL were randomly assigned to one of the three treatment groups (Table 1). There was no significant relationship between length and nadir pressure for any treatment ($P \geq 0.17$). This was done intentionally to reduce the effect of the nadir pressure on the treatment comparisons; i.e., we attempted to ensure fish of all sizes were exposed to the full scope of nadir pressures within the range of interest.

Prior to surgery, all fish were anesthetized with tricaine methanesulfonate (MS-222; 80 mg/L) buffered with sodium bicarbonate (80 mg/L) to stage 4 anesthesia (loss of equilibrium, reflexes, and muscle tone with a slow but steady opercular rate: Summerfelt and Smith 1990). For fish receiving an AT-only or AT+PIT, disinfected transmitters (submersed in 70% ethanol for 20 min and rinsed in sterile water) were inserted by hand through a 3-mm incision made with a sterile, number 11 surgical blade approximately 2–3 mm above the linea alba (where the tip of the pectoral fin lies against the right side of the fish's body). To minimize potential loss of tags, the PIT tag, when used, was always inserted first and both tags were massaged away from the incision. Untagged fish were handled similarly, i.e., were anesthetized and held out of water for a similar time as fish undergoing surgery (<60 s), to minimize handling bias.

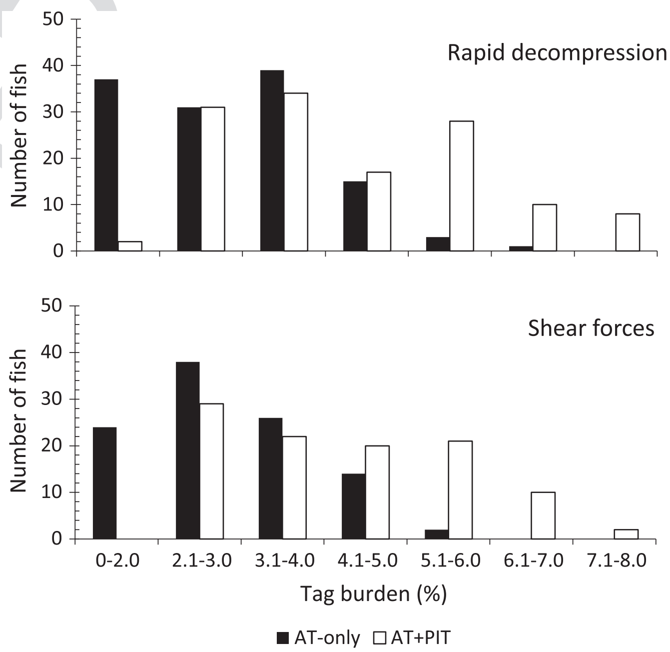

FIGURE 2. The tag burdens (weight of transmitter as a percentage of the weight of the fish) were evenly distributed between exposure tests of rapid decompression (top panel) and shear forces (bottom panel) but did increase in fish given both an acoustic transmitter and PIT tag (AT+PIT) relative to fish given only an acoustic transmitter (AT-only).

Fish were allowed to recover overnight (16 to 24 h) and then placed into hyper–hypobaric chambers to acclimate (six to nine fish per chamber). Once in the chambers, fish were acclimated to 222 kPa absolute (kPaA) (32.2 psi absolute [psia]) for 16 to 24 h. This pressure value represented a depth of approximately 12 m, which was found to be the median acclimation depth of turbine-passed, acoustic-tagged, subyearling Chinook Salmon at Little Goose Dam on the Snake River, Washington, in the summer of 2013 (Li et al. 2015). Immediately upon completion of the acclimation period, fish behavior was evaluated to ensure test fish were in a state of neutral buoyancy (i.e., maintaining position within the water column and not resting on the bottom of the chamber; Perry et al. 2001; Stephenson et al. 2010), and then fish were exposed to a nadir pressure of approximately 69–124 kPaA (10–18 psia) with a rate of change approximately matching a fish's experience during actual turbine passage. We selected this range of nadir pressures to simulate the nadir pressures that were closest to the pressure measured during sensor fish releases through turbines during operations at Ice Harbor Dam on the Snake River (Z. D. Deng, unpublished data). The nadir and acclimation pressures used in this study resulted in a mean log ratio pressure change of all three groups equal to 0.85 (range, 0.61–1.15).

Immediately following pressure exposure, all fish were euthanized (if not dead from exposure) and necropsied as described in Stephenson et al. (2010). Briefly, each fish was examined for transmitter or viscera expulsion; swim bladder condition (ruptured, intact, or hemorrhaged); exophthalmia and other signs of external hemorrhaging, emphysema or embolisms in fins; signs of internal organ damage (blood, bruising, or hemorrhaging); condition and openness of incision; and location of transmitter(s). The mortal injury index described by McKinstry et al. (2007) was used to classify dead fish and fish with sufficient injuries to cause death even though they were alive following pressure exposure. Fish were assigned a mortal injury if at least one of the following conditions was found during necropsy: greater than mild embolism in the gills, emphysema in a pelvic fin, blood from the vent, rupture of any part of the swim bladder, internal hemorrhaging in the kidney, liver, or heart, and any visceral or transmitter protruding from the incision site.

Exposure to shear forces.— Exposure to shear forces was performed in the shear tank located at PNNL using methods previously described (Neitzel et al. 2004; Deng et al. 2005, 2010). On 5 d between October 11 and October 26, 2016, a total of 308 fish in the size range of 70 to 106 mm FL were randomly assigned to one of the three treatment groups (Table 1). Fish were tagged, or in the case of untagged fish, handled in a similar manner as tagged fish, following the surgical protocols from the rapid decompression study. Fish were allowed to recover from surgery overnight (16 to 24 h) in a recovery tank. Fish were then removed from the recovery tank and directly introduced to a high-pressure jet of water through a polycarbonate introduction tube (60 cm long, 3.18 cm diameter) that was positioned immediately above a conical stainless-steel nozzle (beginning diameter of 25.4 cm, constricted to 6.35 cm diameter over a length of 50.8 cm) that delivered water into the shear tank (9 m long, 1.2 m wide, and 1.2 m deep) at a velocity of 12 m/s. Nozzle velocities were created using a centrifugal pump with a programmable electronic speed controller; a flow conditioner was incorporated into the delivery system to reduce inlet turbulence. This nozzle velocity is the jet velocity at which a difference in injury rates between untagged fish and fish tagged with external transmitters was observed in previous research and is also representative of shear forces fish may encounter during dam passage in the FCRPS (Deng et al. 2012). The duration of exposure (<1 s) was consistent with the durations of significant shear forces measured during sensor fish releases through the turbines during the operation of hydropower dams in the Columbia River basin (PNNL, unpublished data). While we recognize that juvenile salmon migrating downstream through the hydropower system could experience rapid decompression and shear forces at the same time, we were not able to expose a fish to a rapid jet of water in our hyperbaric chamber nor were we able to pressurize our shear tank to expose them to rapid decompression.

Within about 10 s following each individual exposure, the pump was turned off and each fish was netted from the shear tank. Swimming behavior (normal, loss of equilibrium, lethargic, or erratic) was evaluated based on the swimming behavior during recapture. Once fish were captured, they were placed within a section of clear tubing containing a small volume of water and examined for survival, transmitter or visceral expulsion, and external injuries (e.g., ripped fins, bulging or otherwise damaged eyes, hemorrhaging, bleeding, or descaling). All surviving fish were held in a separate recovery tank for up to 48 h and examined twice for survival, transmitter or visceral expulsion, and external injuries (those noted above plus dark discoloration, lethargy, and equilibrium loss), once after 16 to 24 h postexposure and again at 40 to 48 h postexposure (Guensch et al. 2002). All external injuries were considered mortal injuries because they are indicative of other internal injuries (Neitzel et al. 2000, 2004).

Statistical analyses.— The proportion of fish that survived after exposure to rapid decompression included fish that survived the pressure exposure without exhibiting a mortal injury. The proportion of fish that survived exposure to shear forces included fish that survived for at least 2 d postexposure without a mortal injury. Fisher's exact tests were used to determine whether the proportion of fish that survived differed significantly among treatments.

The effect of the transmitter on the performance of tagged fish exposed to simulated turbine passage was evaluated using generalized linear models (GLMs) that

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incorporate a logit link function and Bernoulli error structure. Applying this approach to the survival data was determined to be appropriate by evaluating Pearson goodness-of-fit (GOF) test results for all models ($\chi^2 \leq 272.041$, $P \geq 0.437$). Models were evaluated using Akaike's information criterion corrected for small sample sizes (AIC_c) and likelihood ratio tests (LRTs). The best-fitting model was identified as the model with the lowest AIC_c that significantly improved the fit over nested models containing fewer parameters as indicated by LRTs. In addition, the predictive potential of each model was evaluated using the area under the curve (AUC) metric (Hosmer and Lemeshow 2000).

The effect of tag burden on survival was evaluated by first comparing a reduced, intercept-only model to a model that included terms for the intercept and tag burden (equation 1):

$$S = \frac{1}{1 + \exp(-(\beta_0 + \beta_1 B))}, \tag{1}$$

where the estimated survival probability (S) is represented as a logistic function with intercept β_0 and slope β_1 fit to the binomial survival data, where fish that survive as a result of simulated turbine passage were assigned a 1 or otherwise a 0, and B is the tag burden measured as a proportion. We then compared survival of treatment groups with untagged fish for each stressor using methods similar to those described by Perry et al. (2013) in an attempt to identify a threshold value at which tagged and untagged fish performed similarly. That is, a treatment effect was included in the GLM fit to the survival data of tagged and untagged fish:

$$S = \frac{1}{1 + \exp(-(\beta_0 + \beta_1 B + I_T \beta_2))}, \tag{2}$$

where parameters were as described in equation (1) with the addition of I_T, an indicator variable resolving to 1 for tagged fish and 0 for untagged fish, and β_2, which estimates the difference in intercepts between tagged and untagged fish. Negative estimates of β_2 indicate tagged fish performed worse than untagged fish over the full range of tag burdens, whereas positive estimates of β_2 support the hypothesis that a tag burden threshold exists, below which tagged and untagged fish perform similarly. The tag burden threshold can be estimated by setting S equal to the proportion of untagged fish that survived exposure to simulated turbine passage stressors and iteratively solving for B. The full model shown in equation (2) was compared with the model shown in equation (1) using AIC_c, LRTs, and AUC to determine whether inclusion of the β_2 term improved the fit and predictive ability of the model, thus providing support for the presence of a tag burden threshold.

This approach assumes survival of untagged fish is constant with respect to mass because the effect of the transmitter is estimated from the observed proportion of the untagged group that survived simulated turbine passage. To test this assumption, logistic regression models were fit to the binomial survival data for untagged fish exposed to simulated turbine passage stressors as a function of fish weight. This model was then compared with a model that included only an intercept term using LRTs to evaluate the effect of fish weight on survival. The inclusion of weight as a predictor of survival for untagged fish exposed to rapid decompression did not represent an improvement over the intercept-only model (LRT $\chi^2 = 0.001$, $P = 0.975$). However, the model that included weight as a predictor of survival for untagged fish exposed to shear forces provided a better fit than the intercept-only model (LRT $\chi^2 = 7.369$, $P = 0.007$). Untagged fish that weighed ≤5.1 g (~73 mm FL) were more likely to suffer mortality than fish that weighed >5.1 g when exposed to shear forces. To ameliorate this effect, all tagged ($n = 10$ AT-only and $n = 8$ AT+PIT) fish and untagged ($n = 7$) fish that weighed ≤5.1 g were removed from the shear force data set before evaluating the effect of the transmitter on survival of tagged fish as described above. All statistical analyses were performed using a significance levels (α) set at 0.05.

RESULTS

Exposure to Rapid Decompression

The proportion of untagged fish that survived was significantly higher than the proportion surviving in the AT-only group ($P = 0.005$) and in the AT+PIT group ($P < 0.001$) (Table 2). Survival in the AT+PIT group was significantly lower than in the AT-only group ($P = 0.01$). No ATs or PIT tags were fully expelled during rapid decompression, although three tested fish in the AT+PIT group were found to have at least one partially protruded tag, and 11 tested fish in the AT-only group and 26 tested fish in the AT+PIT group were found with partially protruding viscera (Table 2). A ruptured swim bladder was noted in 15.5% of the untagged fish tested, 26.0% of the AT-only fish tested, and 33.8% of the AT+PIT fish tested; swim bladder rupture accounted for 83% of all mortal injuries of fish exposed to rapid decompression (Table 2). Other mortal injuries noted included blood in the vent, internal hemorrhaging of the liver, kidney, or heart, and undetermined mortality (Table 2).

Tagged fish were more susceptible to injury or death from rapid decompression than untagged fish over the range of tag burdens tested (Figure 3A, B). The model

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that included tag burden as a predictor variable provided a substantially better fit to the survival data than did the reduced, intercept-only model for AT-only (LRT $\chi^2 = 7.996$, $P = 0.005$) and AT+PIT (LRT $\chi^2 = 34.916$, $P < 0.001$). The model containing the treatment effect (equation 2) was not significantly better at predicting fish survival than the model described in equation (1) for either AT-only (LRT $\chi^2 = 0.290$, $P = 0.591$) or AT+PIT (LRT $\chi^2 = 0.625$, $P = 0.429$) test fish, providing little evidence of a tag burden threshold for tagged fish exposed to rapid decompression. Additionally, estimates of β_2 were negative for AT-only and AT+PIT groups indicating no tag burden threshold existed below which tagged and untagged fish had a similar probability of survival during exposure to rapid decompression (Table 3). Therefore, the model described above in equation (1) provides the best representation of the transmitter effect on survival of fish exposed to rapid decompression (Figure 3A, B). Predicted survival of juvenile Chinook Salmon implanted with an acoustic transmitter and exposed to rapid decompression would range from 0.588 for fish measuring 70 mm FL (tag burden, 5.1%) to 0.791 for fish measuring 110 mm FL (tag burden, 1.3%; Table 4). The addition of a PIT tag further reduced survival (Table 4).

Exposure to Shear Forces

Significantly fewer tagged fish survived exposure to shear forces than untagged fish (AT-only versus untagged: $P \leq 0.001$; AT+PIT versus untagged: $P \leq 0.001$) (Table 2). However, the difference between the AT-only group and the AT+PIT group was not significant ($P = 0.19$). One fish in the AT-only group and three fish in the AT+PIT group expelled at least one of their tags when exposed to shear forces (Table 2). Most of the mortalities in both groups were due to viscera protrusion (Table 2). Viscera protrusion was noted in 10 AT-only and 18 AT+PIT fish before they were exposed to shear forces, but only two of the AT-only and six of the AT+PIT fish were counted as mortalities because the severity of the protrusion worsened in these fish after the tests.

Similar to the results from the rapid decompression tests, tagged fish exposed to shear forces were more susceptible to injury or death from shear forces than were untagged fish over the full range of tag burdens (Figure 3C, D). The model that included tag burden as a predictor variable provided a substantially better fit to the survival data than the reduced, intercept-only model for AT-only (LRT $\chi^2 = 37.476$, $P < 0.001$) and AT+PIT (LRT $\chi^2 = 71.364$, $P < 0.001$) groups. The model containing the treatment effect (equation 2) was not significantly better at predicting fish survival than the model described in equation (1) for either AT-only (LRT $\chi^2 = 2.129$, $P = 0.145$) or AT+PIT (LRT $\chi^2 = 0.863$, $P = 0.353$) fish, providing little evidence of a tag burden threshold for tagged fish exposed to shear forces. Additionally, estimates of β_2 were negative for both AT-only and AT+PIT groups (Table 3) indicating no tag burden threshold existed below which tagged and untagged fish had a similar probability of survival during exposure to shear forces. Therefore, just as with the rapid decompression tests, the model described above in equation (1) provides the best representation of the transmitter effect on survival of fish exposed to shear forces (Figure 3C, D). Predicted survival of juvenile Chinook Salmon (>5.1 g) implanted with an acoustic transmitter and exposed to shear forces would range from 0.388 for fish measuring 75 mm FL (tag burden, 4.3%) to 0.955 for fish measuring 110 mm FL (tag burden, 1.3%; Table 4). The addition of a PIT tag further reduced survival (Table 4).

TABLE 2. The number of juvenile Chinook Salmon that died or were mortally injured following exposure to rapid decompression or shear forces. Treatments were untagged controls, acoustic transmitter (AT-only), and acoustic transmitter and a PIT tag (AT+PIT). n = the number of fish tested in each treatment, S = the proportion surviving within each treatment, and M_{total} = the total number of fish counted as a mortality, which included fish with protruded (rapid decompression) or fully expelled (shear forces) tags, protruded viscera, ruptured swim bladder, and/or blood in their vent, showed signs of internal hemorrhaging in the liver, kidney or heart, or died from unknown causes. Note that fish may be included in more than one mortality category, so columns do not always sum to the total mortality. ND = no data were collected.

Treatment	n	S	M_{total}	Protruded or expelled tags	Protruded viscera	Ruptured swim bladder	Blood in vent	Internal hemorrhage	Unknown mortality
Rapid decompression									
Untagged	142	0.845	22			22	0	1	0
AT-only	127	0.701	38	0	11	33	1	0	2
AT+PIT	130	0.538	60	3	26	44	4	4	2
Shear forces									
Untagged	100	0.980	2			ND	ND	ND	2
AT-only	104	0.702	31	1	28	ND	ND	ND	3
AT+PIT	104	0.606	41	3	39	ND	ND	ND	0

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LOW RESOLUTION FIG

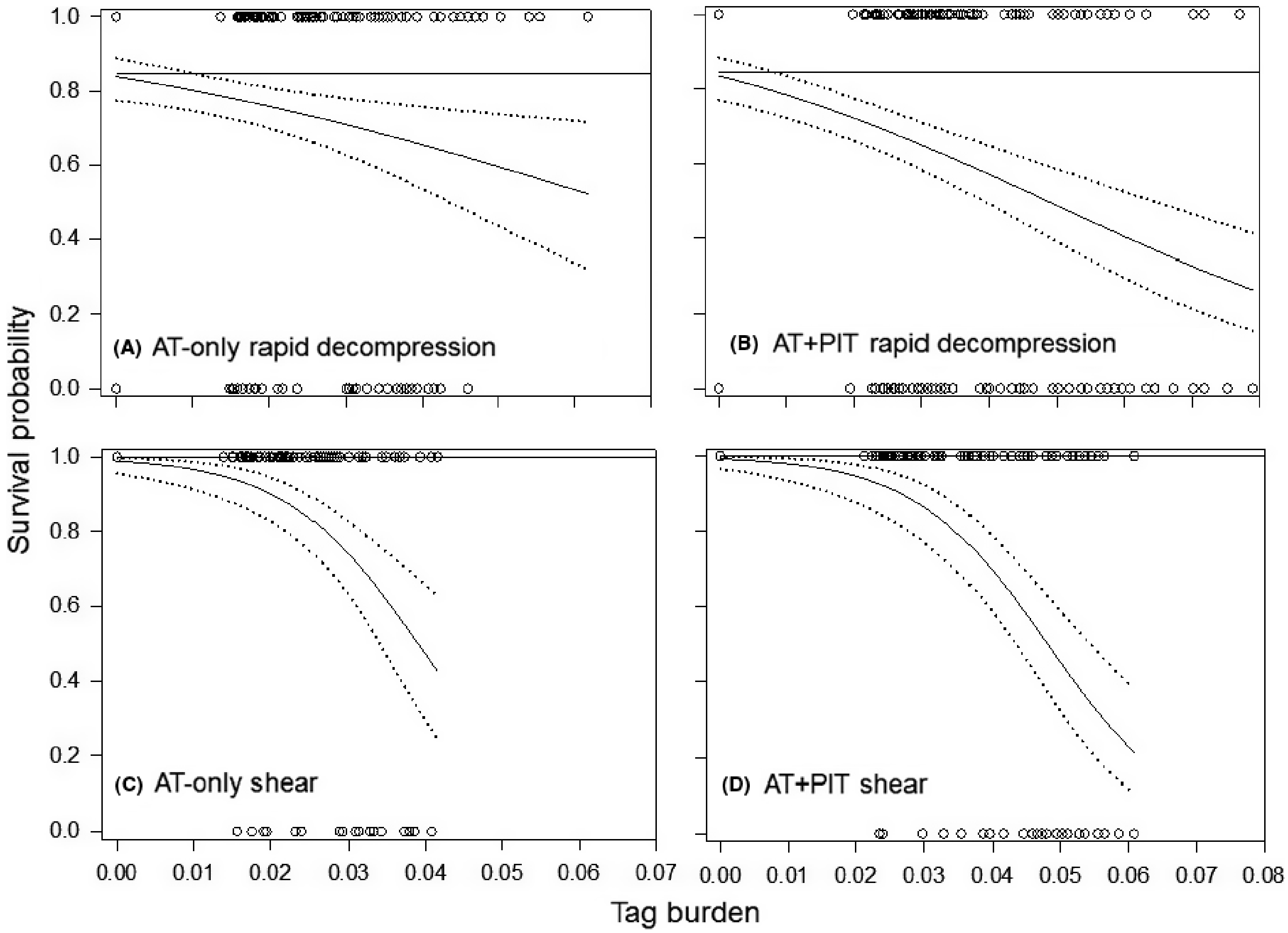

FIGURE 3. Logistic regression model relationships between tag burden and survival probability for **(A)** AT-only fish exposed to rapid decompression, **(B)** AT+PIT fish exposed to rapid decompression, **(C)** AT-only fish exposed to shear forces, and **(D)** AT+PIT fish exposed to shear forces. Dotted lines around the modeled relationships represent 95% confidence intervals. Thin solid horizontal lines at 0.845 on *y*-axis for panels (A) and (B) and at 1.0 on *y*-axis for panels (C) and (D) represent the proportion of untagged control fish that survived exposure to simulated turbine passage stressors. Open dots show the fate (1 = survived, 0 = died) of each individual fish exposed to simulated turbine passage stressors.

DISCUSSION

This study used a series of experiments designed to replicate the rapid decompression and shear forces that juvenile salmon could experience as they migrate downstream past hydroturbines and over spillways in the FCRPS. Our objective was to determine whether a new, cylindrical, microacoustic transmitter could be implanted in juvenile Chinook Salmon smaller than 95 mm FL, the current minimum size threshold used to conduct survival studies in the Columbia River basin and widely used for field research studies. The results showed that tagged fish were more susceptible to stressors than untagged fish over the range of tag burdens tested, and we observed that as tag burden increased, survival decreased. However, the selection of a single minimum threshold for tagging with the new microacoustic transmitter using an unsutured incision was complicated by the variability in our study results and the lack of a tag burden threshold. Without such a threshold, we are limited in our ability to recommend a revised minimum length of Chinook Salmon at which the new microacoustic transmitter will not have an effect on survival when the fish are exposed to rapid decompression and shear forces. Consequently, we recommend that a conservative minimum size threshold continue to be 95 mm FL and that a 3-mm incision be made above the linea alba with no sutures.

Other laboratory studies evaluating tag effects with the same acoustic transmitter in Chinook Salmon showed mixed results in determining tagging thresholds. Walker et al. (2016) suggested a minimum size threshold of 79 mm FL, while Liss et al. (2016) could find no significant difference in 60-d survival rates among juvenile

TABLE 3. Results of generalized linear models fit to the binomial survival data of tagged and untagged juvenile Chinook Salmon exposed to simulated turbine passage stressors as a function of intercept (β_0), tag burden ($\beta_1 B$), and tag burden threshold ($I_T\beta_2$) terms. Standard errors of variable coefficients are shown in parentheses. n_T = tagged fish sample size, n_C = untagged fish sample size, S_C = proportion of untagged fish that survived stressor exposure, AIC_c = Akaike information criterion corrected for small sample sizes, AUC = area under the curve.

Model	n_T	n_C	S_C	β_0	β_1	β_2	AIC_c	AUC
				AT-only rapid decompression				
β_0	126	142	0.845	1.27 (0.15)			286.6	0.50
$\beta_0 + \beta_1 B$	126	142	0.845	1.65 (0.21)	−25.28 (8.93)		280.6	0.62
$\beta_0 + \beta_1 B + I_T\beta_2$	126	142	0.845	1.53 (0.31)	−16.439 (18.70)	−0.17 (0.31)	282.4	0.62
				AT+PIT rapid decompression				
β_0	130	142	0.845	0.84 (0.13)			337.0	0.50
$\beta_0 + \beta_1 B$	130	142	0.845	1.63 (0.21)	−33.55 (5.95)		304.2	0.71
$\beta_0 + \beta_1 B + I_T\beta_2$	130	142	0.845	1.46 (0.30)	−25.12 (12.10)	−0.24 (0.30)	305.6	0.71
				AT-only shear				
β_0	93	93	1.000	2.01 (0.23)			139.3	0.50
$\beta_0 + \beta_1 B$	93	93	1.000	4.53 (0.74)	−116.13 (24.74)		103.9	0.86
$\beta_0 + \beta_1 B + I_T\beta_2$	93	93	1.000	4.30 (0.86)	−80.06 (31.90)	−0.93 (0.86)	103.9	0.86
				AT+PIT shear				
β_0	96	93	1.000	1.52 (0.19)			182.2	0.50
$\beta_0 + \beta_1 B$	96	93	1.000	4.93 (0.80)	−102.53 (18.17)		112.8	0.91
$\beta_0 + \beta_1 B + I_T\beta_2$	96	93	1.000	4.67 (0.86)	−84.64 (21.52)	−0.56 (0.86)	114.1	0.91

TABLE 4. Predicted tag burdens (%) and survival proportions over a range of fork lengths between 70 and 110 mm for juvenile Chinook Salmon tagged with an acoustic transmitter (AT-only) or an acoustic transmitter and a PIT tag (AT+PIT). Tag burdens (B) were predicted using exponential relationships between FL and tag burden for AT-only ($B = 59.26^{(-0.035 \times FL)}$) and AT+PIT ($B = 83.70^{(-0.035 \times FL)}$). Survival values were predicted using a logistic regression model (equation 1) that incorporated tag burdens and the parameters from Table 3. For reference, survival of untagged fish exposed to rapid decompression was 0.845 and for shear forces (only fish > 5.1 g, ~73 mm FL) was 1.0.

FL (mm) Treatment	70	75	80	85	90	95	100	105	110
				Predicted tag burden (%)					
AT-only	5.1	4.3	3.6	3.0	2.5	2.1	1.8	1.5	1.3
AT+PIT	7.2	6.1	5.1	4.3	3.6	3.0	2.5	2.1	1.8
				Predicted survival for rapid decompression					
AT-only	0.588	0.638	0.677	0.708	0.733	0.752	0.768	0.781	0.791
AT+PIT	0.311	0.400	0.481	0.549	0.605	0.650	0.686	0.715	0.737
				Predicted survival for shear					
AT-only	NA[a]	0.388	0.585	0.734	0.829	0.886	0.921	0.942	0.955
AT+PIT	NA[a]	0.216	0.428	0.634	0.778	0.863	0.912	0.940	0.957

[a]Survival of untagged fish was not constant at weights ≤ 5.1 g (~73 mm FL), so these fish were removed from the model analysis of the shear force data set.

Chinook Salmon of 65–104 mm FL. In a separate study (PNNL, unpublished data), similar-sized juvenile Chinook Salmon (65–104 mm FL, as used by Liss et al. 2016) were implanted with the new microacoustic transmitter; however, the unpublished study used an unsutured incision instead of the injection technique that was used by Liss et al. (2016). Fish were again held in the laboratory for 60 d, and logistic regression analysis of the survival data indicated that the minimum size for tagging would be 83 mm FL for AT-only (mean tag burden, 3.2%) and 79 mm FL for AT+PIT (mean tag burden, 5.7%) fish. However, 79 mm FL was near the minimum size of fish that were tested, so there was considerable uncertainty around this estimate (PNNL, unpublished data).

Field studies indicated improvement in survival using the newer, smaller transmitter compared with tests conducted with a larger acoustic transmitter. Subyearling Chinook Salmon (95–143 mm FL) injected with the

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microacoustic transmitter had a survival probability of 0.26 (SE = 0.02) over 500 km within the Snake and Columbia rivers compared with a survival probability of 0.20 (SE = 0.01) for similar-sized Chinook Salmon implanted with a larger, commercially available, JSATS transmitter (length, 12 mm; width, 5.2 mm; height, 3.8 mm; weight in air, 0.43 g); the differences were significant ($P = 0.002$) (Deng et al. 2017). Deng et al. (2017) believed that the reduction in transmitter size reduced the "tag-effect" and the use of an implantation method without sutures reduced the "tagging-effect," both of which improved survival. In another study, subyearling Chinook Salmon (80–103 mm FL) were surgically implanted (3-mm incision, no sutures) with either an AT+PIT or a PIT-only and monitored for survival as they migrated from a hatchery to the Columbia River and then again as they migrated 165 km downstream to McNary Dam (Harnish et al. 2014). Survival probability of fish from the hatchery to the Columbia River in the AT+PIT group was approximately 0.82, which was significantly lower than the survival probability in the PIT-only group ($S = 0.92$; LRT $\chi^2 = 17.077$, $P < 0.001$), suggesting a tag or tagging effect contributed to mortality in the AT+PIT group. Further, even though detection rates of both systems were quite high (>96%), about 5% of the fish given an AT+PIT tag appeared to drop the AT within 2 weeks posttagging as evidenced by their detection by the PIT array but not the AT array. Survival in the AT+PIT group appeared to be related to fish length, and modeled survival probabilities of the two groups converged at 99 mm FL. Survival probability from the hatchery outlet to McNary Dam was approximately 0.53 for the AT+PIT group and 0.63 for the PIT-only group; survival values were not significantly different (LRT $\chi^2 = 2.318$, $P = 0.128$). Although these differences were not significant, about 7% of the fish appeared to drop their AT before reaching McNary Dam as evidenced by PIT detections in the McNary Dam juvenile bypass system but not on an adjacent AT array. This mortality was related to fish size with a convergence of survival probabilities at around 94 mm FL.

In the current study ruptured swim bladders in fish exposed to rapid decompression were the primary cause of mortal injury in all test fish, and fish with an acoustic transmitter or an acoustic transmitter and PIT tag were about twice as likely to have a ruptured swim bladder as untagged fish. In general, acoustic transmitters and PIT tags have the potential to negatively affect juvenile salmonid survival when tag burdens exceed 6.7% (Chittenden et al. 2009; Brown et al. 2010). The presence of a telemetry device has also been associated with increased mortality and injury in fish exposed to rapid decompression that simulated hydroturbine passage even at tag burdens much less than this amount (Brown et al. 2009; Carlson et al. 2012). When juvenile Chinook Salmon were exposed to pressure changes that simulated passage through a Kaplan-type turbine, fish implanted with a radio transmitter (tag burdens, 1.3–4.7%) suffered higher mortality and injury than untagged fish (Brown et al. 2009). The severity of mortality and injury depended on the method of transmitter implantation, the depth of acclimation, the nadir pressure, and the size of the fish. Using acoustic transmitters and PIT tags, Carlson et al. (2012) examined a wider range of tag burdens (0–6.6%; both PIT tags and AT) than did Brown et al. (2009) and found that, other factors being the same, as the tag burden increased the rate of mortal injury in juvenile Chinook Salmon also increased after exposure to simulated turbine passage. The increase in mortal injury during simulated turbine passage at higher tag burdens is likely because the presence of a telemetry device amplifies the swim bladder hyperinflation due to rapid decompression (Brown et al. 2009). To achieve neutral buoyancy, fish can compensate for the increase of their weight in water from a telemetry device by increasing the volume of their swim bladder (Gallepp and Magnuson 1972; Perry et al. 2001). Rapid decompression like that which occurs during turbine passage may result in an expansion of the gas inside the swim bladder at a rate that is higher than what the swim bladder tissue can adjust for (Pflugrath et al. 2012). In addition, the transmitter also reduces the abdominal volume available to accommodate the swim bladder during rapid decompression, resulting in high pressures on internal organs and increased barotrauma injuries (Brown et al. 2009). A ruptured swim bladder can make it difficult for the fish to maintain neutral buoyancy and orientation, which in turn would likely lead to an increase in their susceptibility to predation. As such, swim bladder rupture, like that found in our study, is the most common mortal injury found in rapid decompression associated with the pressure changes that occur in turbine passage.

In addition to tag burden, the change in pressure from acclimation to exposure is a significant factor in predicting the likelihood of barotrauma injuries for fish exposed to rapid decompression (Brown et al. 2009, 2012; Carlson et al. 2012). At the mean log ratio pressure change value used in our study (0.85), the probability of mortal injury predicted by Carlson et al. (2012; equation 1) in the AT-only group (mean tag burden, 2.9%) would have been approximately 0.31, and in the AT+PIT group (mean tag burden, 4.2%) this value would have been approximately 0.50. Our actual probabilities of mortal injury values were 0.30 and 0.46 for AT-only and AT+PIT groups, respectively, which were very similar to the predicted values.

Similar to our findings, Johnson et al. (2003) showed that untagged juvenile Chinook Salmon (87–100 mm FL) were not injured or killed when exposed to shear velocities of less than 15.2 m/s. Rainbow Trout (mean = 120 mm

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FL) were more susceptible to predation at shear velocities of 9.1–12.2 m/s, but minor and major injuries became significant only after shear velocities were greater than 15.2 and 18.3 m/s, respectively (Neitzel et al. 2000). The onset of minor, major, and fatal injuries to juvenile Chinook Salmon (93–128 mm FL) occurred at jet velocities of 12.2, 13.7, and 16.8 m/s, respectively, and the most common injuries were to the operculum, and those occurred at jet velocities around 12 m/s (Deng et al. 2005). Comparisons are limited, however, because no other studies have been conducted on the effects of internal tags on survival of juvenile salmonids exposed to shear.

No transmitters or PIT tags were expelled from the fish during rapid decompression testing, but posttest examinations of live fish showed transmitters or viscera protruded from the incision sites. Transmitter or visceral expulsion in the tagged groups was the primary cause of fish being assigned a mortal injury when exposed to shear velocities. Even though the wound area created by the incision method we used was small, the lack of sutures resulted in both viscera and transmitters being expelled or protruding from the incision following exposure to rapid decompression and shear velocities. Although our incisions and transmitters were smaller than those studied by Boyd et al. (2011) and our tag burdens were relatively low compared with many other studies (Brown et al. 2010; Carlson et al. 2012), it is apparent that the dynamic environment of hydropower dam passage creates conditions that make the use of an unsutured incision less likely to retain viscera and transmitters or PIT tags.

The protrusion of viscera may have been influenced by the fact that our test fish were noted during necropsies to have an abundance of fatty tissue in the peritoneal cavity. Although not measured, we assumed that the presence of this tissue would have reduced the space in the peritoneal cavity that otherwise would have been open and available for the swim bladder to expand into during rapid decompression. The fatty tissue may have also exacerbated visceral expulsion during both rapid decompression and shear experiments. In fact, even prior to exposure to shear, 10% to 17% of the fish in the tagged groups were noted to have viscera protruding from the incision, which, in theory, would have increased the likelihood of visceral expulsion during the tests. Our observation from previous telemetry studies of Chinook Salmon is that fatty tissue in the peritoneal cavity is more prevalent in hatchery fish that are well fed and larger than wild fish. As such, the occurrence of fatty tissue in the peritoneal cavity should be considered in future studies using hatchery fish. We also know that the incidence of visceral protrusion increased as fish got smaller, presumably because internal pressure on the viscera increased with the presence of transmitters. This pressure was even higher in increasingly smaller fish that have proportionally smaller body cavities. The presence of both a transmitter and a PIT tag in the body cavity appeared to increase the risk of mortality as evidenced by survival rates of fish in the AT-only group being significantly higher than for fish in the AT+PIT group. Therefore, even though tag burden values were well below the recommendations of most tagging studies (Brown et al. 2010), the volume of the telemetry devices may have been too high relative to the volume of the body cavity and would explain why fish with devices in their body cavities experienced more swim bladder ruptures and protruding viscera.

Management Implications

Even though our recommendation of a minimum tagging length is not a change from the minimum size threshold for Chinook Salmon now currently employed in the Columbia River basin, the new microacoustic transmitter represents a reduction in tag burden from previous versions of the JSATS transmitter by approximately 30%, which is predicted to improve the probability of fish surviving hydroturbine passage. For example, the tag burden of a 95-mm-FL (11.0 g) Chinook Salmon used in our study would be 2.1% using the new microacoustic transmitter (weight in air, 0.22 g) and 3.2% using the previous version of the JSATS transmitter (weight in air, 0.35 g). Using a log ratio pressure change value of 0.85 and the two tag burdens and equation (1) in Carlson et al. (2012), we estimate that the probability of survival would increase from 0.622 with the previous JSATS transmitter to 0.758 with the new microacoustic transmitter for a 95-mm-FL Chinook Salmon. Using the current surgical protocols (i.e., two sutures to close the 5–6-mm incision; USACE 2011), surgery takes ~2–2.5 min. For comparison, the unsutured incision method takes <60 s (Cook et al. 2014). Shorter surgery times result in fish undergoing less handling and less time on the surgery table, which may reduce bias of survival estimates (Deng et al. 2017). Thus, the use of the new microacoustic transmitter is expected to reduce the negative bias of survival estimates associated with turbine passage.

Hydropower development has the potential to expand as demand for power increases (USDOE 2016). Fisheries and water power management agencies continue to seek a balanced approach for operating hydropower facilities in rivers such as the Columbia River that have sensitive aquatic species. The infrastructure associated with hydropower development (i.e., turbine intake devices, spillways, bypass facilities, etc.) creates physical conditions that result in significant and rapid changes in the pressure and shear forces that fish experience as they migrate downstream in rivers where this development has occurred. Monitoring fish populations in rivers with hydropower development will continue to rely on telemetry. It is imperative that the telemetry device implanted into the

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fish does not negatively affect fish performance. The availability of smaller transmitters, such as the new cylindrical microacoustic transmitter, represents a potentially significant contribution to that endeavor. Future studies of fish passage, in which fish could be exposed to rapid decompression and shear forces, should use the smallest tag possible—both in volume and mass—to minimize bias. Additional research on neutrally buoyant transmitters (Deng et al. 2012) is recommended for use in future turbine passage studies.

ACKNOWLEDGMENTS

This research was funded by the U.S. Army Corps of Engineers (USACE), Portland District; Scott Fielding and Brad Eppard were the contracting officers from the USACE. We sincerely thank the Washington Department of Fish and Wildlife Leavenworth Hatchery for providing Chinook Salmon eggs. We appreciate the support and contributions of the staff at PNNL, including Tim Linley and Jill Janak for their assistance in fish husbandry and laboratory use and Alison Colotelo, Kris Hand, and Briana Rhode for their aid with data collection, and reviewers of this manuscript for useful suggestions. All work with fish was reviewed and approved by the PNNL's Institutional Animal Care and Use Committee (IACUC protocol numbers 2014-07 and 2015-01). The PNNL is operated by Battelle for the U.S. Department of Energy under contract number DE-AC05-76RL01830. Reference to trade names does not imply endorsement by the U.S. Government, and the opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army. There is no conflict of interest declared in this article.

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