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# The Influence of Tag Presence on the Mortality of Juvenile Chinook Salmon Exposed to Simulated Hydroturbine Passage: Implications for Survival Estimates and Management of Hydroelectric Facilities

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## ARTICLE

# The Influence of Tag Presence on the Mortality of Juvenile Chinook Salmon Exposed to Simulated Hydroturbine Passage: Implications for Survival Estimates and Management of Hydroelectric Facilities

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#### Abstract

Each year, telemetry tags (acoustic, radio, and passive integrated transponder tags) are surgically implanted into thousands of fish to assess their passage and survival through hydropower facilities. One passage route that is of particular concern is through hydroturbines, where fish may be exposed to a range of potential injuries that include barotraumas from rapid decompression. The change in pressure from acclimation to exposure (nadir) has been identified as an important factor in predicting the likelihood of mortality and injury for juvenile Chinook salmon Oncorhynchus tshawytscha undergoing rapid decompression associated with simulated turbine passage. The presence of telemetry tags has also been shown to influence the likelihood of mortality and injury for juvenile Chinook salmon. We investigated the likelihood of mortality and injury for telemetry-tagged juvenile Chinook salmon that were exposed to a range of pressure changes associated with simulated turbine passage. Several factors were examined as predictors of mortal injury for fish undergoing rapid decompression; of these factors, the loge transformed ratio of acclimation pressure : exposure pressure (LRP) and the tag burden (tag mass expressed as a percentage of fish mass) were the most predictive. As the LRP and tag burden increased, the likelihood of mortal injury also increased. Our results suggest that previous estimates of survival for juvenile Chinook salmon passing through hydroturbines were negatively biased due to the presence of telemetry tags, and this has direct implications for the management of hydroelectric facilities. Realistic examples indicate how the bias in turbine passage survival estimates could be 20% or higher depending on the LRP and tag burden. Negative bias would increase as the tag burden and the pressure change ratio increase and therefore has direct implications for survival estimates. We recommend that future hydroturbine survival studies use the smallest telemetry tags possible to minimize the potential bias associated with tag presence.

\*Corresponding author: rich.brown@pnnl.gov Received January 9, 2011; accepted October 11, 2011 Each year, millions of juvenile salmonids migrate downstream through hydropower-influenced rivers on their seaward migration. Due to the ecological, cultural, and economic importance of salmonids and the Endangered Species Act listing of several stocks, survival rates through hydropower facilities have been a focus of fisheries management and research agencies in the Pacific Northwest and throughout the world. Numerous studies have documented the route (e.g., over spillways, through juvenile bypass facilities, and through turbines) and survival rates of fish passing through hydropower facilities (Bickford and Skalski 2000; Muir et al. 2001; Skalski et al. 2002).

As a way of monitoring passage routes and survival past hydropower facilities, fish are outfitted with telemetry tags. In the Columbia and Snake rivers alone, passive integrated transponder (PIT) tags are implanted into nearly 2 million salmonids each year (McMichael et al. 2010). In addition, thousands of fish are also equipped with acoustic or radio transmitters. One of the main assumptions associated with all tagging studies is that tagged individuals behave in the same manner as untagged individuals (Nielsen 1992; Baras and Lagardère 1995; Bégout Anras et al. 1998). Currently, fish that are fitted with telemetry tags are assumed to have mortality rates similar to those of untagged individuals, and survival estimates are applied based on this assumption. Previous research, however, has suggested that the presence of a tag may influence the survival, growth, and behavior of fish, possibly limiting the inferences that can be made about the general population (Winter 1996; Bridger and Booth 2003; Brown et al. 2010).

One of the hydropower facility passage routes of particular concern for managers and researchers is through hydroturbines (Mathur et al. 1996; Coutant and Whitney 2000; Čada et al. 2006). Passage through hydroturbines may expose individual fish to a variety of sources of injury and mortality, including mechanical strike, cavitation, shear forces, and rapid and extreme pressure changes (Cada 2001). Modifications to turbine design and operating conditions have been studied to limit the occurrence of these sources of injury, but such injuries have not been eliminated (Čada et al. 2006). Although the hydroturbine areas in which fish may be exposed to mechanical strike, cavitation, and shear forces are limited (Coutant and Whitney 2000; Neitzel et al. 2000), rapid decompression and associated barotraumas pose a risk for all fish that pass through turbines. Barotrauma is characterized by the presence of emboli in the gills, damage to the vasculature and the swim bladder, and other injuries (Feathers and Knable 1983; Rummer and Bennett 2005; Brown et al. 2009).

The regulation of gas in the swim bladder allows a fish to maintain neutral buoyancy in the water column. Salmonids are physostomes and can regulate the amount of gas in the swim bladder through the pneumatic duct. To maintain neutral buoyancy below the water surface, a salmonid can increase the volume of gas in its swim bladder by gulping air at the water surface. In addition, a salmonid can expel gas from the swim bladder through its mouth to maintain buoyancy when moving up in the water column. Gases are also found in the blood and tissues of fish. When juvenile salmonids pass through hydroturbines, free gas in the swim bladder expands as the fish are exposed to rapid decompression. Simultaneously, gas dissolved in the blood and tissues may come out of solution and exist as free gas in the fish's vasculature and tissues. During rapid decompression, the change in pressure can be so rapid that a fish may not be able to expel or spit gas from the swim bladder, potentially leading to a ruptured swim bladder (Cramer and Oligher 1964; Feathers and Knable 1983; Rummer and Bennett 2005); in addition, dissolved gases may come out of solution and form bubbles, or emboli (Brown et al. 2009). The formation and increased size of emboli will increase the volume of the blood, thereby increasing intravascular pressure; furthermore, the emboli can cause damage to blood vessels, resulting in hemorrhaging or entry of bubbles into organs, which may compromise organ function. Other barotraumas resulting from rapid decompression can include exophthalmia (pop-eye), hemorrhaging, emboli in the gills, and emboli in the fins (called emphysema), all of which have the potential to impair a fish's behavior and survival (Brown et al. 2009).

The change in pressure from acclimation (the depth at which gases in the body are at equilibrium with their surrounding environment and at which neutral buoyancy has been attained) to exposure (expressed as the ratio of acclimation pressure : exposure pressure) is a significant factor in predicting the likelihood of barotraumas for fish exposed to rapid decompression (Brown et al. 2009, 2012). Because fish that are acclimated to greater depths may have a greater mass of gas present in their swim bladders or dissolved in their tissues and blood, they may be at higher risk for barotraumas resulting from rapid decompression. Brown et al. (2009) showed that as acclimation pressure increased, so did the frequency of barotraumas and mortality for juvenile Chinook salmon Oncorhynchus tshawytscha. However, the Brown et al. (2009) study tested fish over only a narrow range of log<sub>e</sub> transformed ratios of acclimation pressure : exposure pressure (LRPs; 1.7–3.3), and the LRP experienced by fish passing through hydroturbines varies within hydropower facilities depending on fish acclimation depth, turbine design, and operating conditions. To fully understand the influence of hydroturbine passage on fish, it is necessary to investigate the likelihood of barotraumas across a wide range of pressure changes that are representative of those occurring at hydropower facilities.

Another factor that influences the occurrence of barotrauma in hydroturbine-passed fish is the burden associated with carrying a telemetry tag. Brown et al. (2009) showed that fish with transmitters had higher rates of mortality than untagged individuals when exposed to simulated turbine passage (STP). Fish that receive telemetry tags can compensate for the additional mass of the tag by incrementally increasing their displacement over that of an untagged fish of equal mass via increased swim bladder volume (Gallepp and Magnuson 1972; Perry et al. 2001). This increased mass of gas in the swim bladder may put tagged

acoustic tag variations (single- of double-battery tags) and combinations with and whilout a 111 tag were examined.							
Transmitter treatment	п	Number of trials	FL (mm)	Mass (g)	Condition factor		
Double battery + PIT	1,599	245	129 (95–226)	26.2 (8.1–144.7)	1.15 (0.77–2.10)		
Single battery + PIT	1,673	257	133 (95–205)	29.6 (7.9–119.3)	1.17 (0.74–1.64)		
Single battery only	1,859	288	122 (83-204)	20.5 (6.5-114.6)	1.16 (0.85–1.74)		
PIT only	1,826	277	119 (79–212)	19.0 (5.8–117.1)	1.15 (0.88–1.83)		
Untagged	3,785	561	124 (78–205)	20.4 (4.8-134.0)	1.10 (0.65–1.77)		

TABLE 1. Median (range in parentheses) fork length (FL), mass, and condition factor for each treatment group of juvenile Chinook salmon exposed to simulated turbine passage. Treatments represent the different transmitter types and combinations used (acoustic tags and passive integrated transponder [PIT] tags). Two acoustic tag variations (single- or double-battery tags) and combinations with and without a PIT tag were examined.

fish at an increased risk of barotrauma and mortality when exposed to STP. Because a wide size range of juvenile salmonids is exposed to hydroturbine passage and because the tag types and sizes used to study these fish also vary widely, the influence of tag burden (tag mass expressed as a percentage of fish mass) may be highly variable. Due to the paucity of research on this subject, there is a need to investigate how a range of tag burdens affects the likelihood of barotrauma occurring during hydroturbine passage.

The objective of this research was to identify the factors that influence the injuries and mortality of juvenile Chinook salmon with a range of tag burdens and exposed to a range of pressure changes. The examined factors included the LRP, tag burden, tag type, fish length, fish weight, and condition factor. We hypothesized that the risk of mortal injury to telemetrytagged fish during STP would not differ among the types of tags used.

## METHODS

*Experimental fish.*—Subyearling and yearling Chinook salmon were exposed to STP treatments between March 7, 2007, and March 6, 2010, at the Pacific Northwest National Laboratory (PNNL) Aquatic Research Laboratory in Richland, Washington (Table 1). All fish were either acquired as fry or were hatched and reared at the PNNL Aquatic Research Laboratory. Juvenile Chinook salmon were held in 1,100-L circular holding tanks with flow-through ambient well water (17°C) and were nourished with an ad libitum ration of Bio-Diet moist pellets (Bio-Oregon, Longview, Washington).

Implantation of tags.—Fish were netted from a holding tank and were held in a bucket containing approximately 15 L of aerated water. Each fish was then anesthetized in a tricaine methanesulfonate (MS-222) solution (80 mg/L of water) that also contained PolyAqua (0.15 mL/L of water; Kordon Aquarium Products, Hayward, California) until the fish reached stage 4 anesthesia (Summerfelt and Smith 1990). While under anesthesia, fish were measured for fork length (FL; mm) and mass (g). A small portion of the caudal fin was clipped for visual individual identification.

Each fish was then subjected to one of four transmitter treatments: (1) a double-battery acoustic transmitter (a commercially available Juvenile Salmon Acoustic Telemetry System transmitter, Model SS-208; Advanced Telemetry Systems, Isanti, Minnesota) and a PIT tag (Destron Technologies, St. Paul, Minnesota) were surgically implanted into the coelom, (2) a singlebattery acoustic transmitter (a dummy transmitter similar to the double-battery tag but lacking one battery) and a PIT tag, (3) a single-battery acoustic transmitter only, or (4) a PIT tag only, injected into the coelom (Table 2). Double-battery acoustic transmitters were 12.0 mm long, 5.2 mm wide, and 3.8 mm high and weighed 0.43 g. Single-battery acoustic transmitters were 12.0 mm long, 5.2 mm wide, and 3.4 mm high and weighed 0.31 g. The PIT tags were 12.5 mm long and 2.1 mm wide and weighed 0.1 g. In an additional treatment, fish were not fitted with a transmitter but were anesthetized and handled as described above. The double-battery acoustic transmitter + PIT tag treatment and the PIT-tag-only treatment represent the methods currently used in the Columbia and Snake rivers to monitor the survival and passage of fish through hydroelectric facilities. Fish are fitted with two types of tag because the PIT tag

TABLE 2. Combined mass of tags (in air and water), tag volume, and median tag burden (tag weight expressed as a percentage of fish body weight in air; range in parentheses) associated with each transmitter treatment group (defined in Table 1) of juvenile Chinook salmon exposed to simulated turbine passage.

Transmitter treatment	Tag mass in air (g)	Tag mass in water (g)	Tag volume (mL)	Tag burden (%)
Double battery + PIT	0.53	0.36	0.18	2.05 (0.37-6.62)
Single battery $+$ PIT	0.41	0.25	0.15	1.35 (0.34-5.06)
Single battery only	0.31	0.19	0.11	1.49 (0.27-4.69)
PIT only	0.10	0.06	0.04	0.51 (0.08–1.66)

prevents fish from being sorted into transport barges or trucks at juvenile bypass facilities, while the acoustic tag is used to monitor downstream movement. Single-battery acoustic transmitters represent a potential prototype for future studies. Surgeries followed the methodology outlined by Brown et al. (2006). After surgery, fish were placed in 5-L buckets containing oxygenated water and were allowed to recover.

Acclimation prior to pressure exposure and simulated turbine passage.—After fish recovered from surgery (as indicated by the re-establishment of equilibrium and active swimming), they were loaded into the hyperbaric–hypobaric chambers of the Mobile Aquatic Barotrauma Laboratory for acclimation to 4.6m depth (146.1 kPa). This level was chosen because it is near the midpoint of the depth range at which juvenile salmonids can become neutrally buoyant (Pflugrath et al., in press) and because previous research has indicated that barotrauma is associated more with the acclimation pressure : exposure pressure ratio than with acclimation pressure alone (Brown et al. 2009, 2012).

Seven fish were loaded into each chamber and were acclimated for a 16–24-h period (using the methods outlined by Stephenson et al. 2010) to allow ample time for the fish to attain neutral buoyancy and equilibration of gas tensions in bodily fluids and tissues. Buoyancy after this period was determined by using the same observational methods described by Stephenson et al. (2010). Although we tested more than 10,742 fish, a small proportion of these fish never gained neutral buoyancy after 16 h of acclimation (i.e., 4% were negatively buoyant and <0.1% were positively buoyant). Due to the importance of buoyancy prior to rapid decompression exposure (Stephenson et al. 2010) and the assumption that in-river fish are neutrally buoyant when approaching hydroelectric facilities (due to energy conservation), we included only neutrally buoyant fish in the analyses.

Treated water was supplied to all chambers at a continuous rate of 7.6 L/min (flow control accuracy:  $\pm 0.95$  L/min), similar to the conditions described by Stephenson et al. (2010). Ambient well water was supplied to the chambers (median temperature =  $17.0^{\circ}$ C; range =  $15.4-17.9^{\circ}$ C), and the total dissolved gas level was about 115% saturation (median = 115.0%; range = 112.7-118.2%).

*Exposure pressures and rate of pressure change.*—Exposure (nadir) pressures ranged from 9.0 to 200.5 kPa (median = 46.9 kPa; with 101.3 kPa representing surface pressure), and the rate of change during STP ranged from 103.4 to 3,824.6 kPa/s (median = 1,495.4 kPa/s); this would be equivalent to fish undergoing a change in depth at 152.5 m/s. The pressure exposure profiles represented passage through the Kaplan turbine units typical of the lower Snake and Columbia River hydropower projects (see Figure 1 of Brown et al. 2009; Figure 2 of Stephenson et al. 2010) and represented pressure change ratios that juvenile salmon would be expected to experience during hydroturbine passage. The exposure profile simulated the pressure acting on fish that are carried in water passing through the turbines, which may increase to approximately 400 kPa over a period of about 20 s as fish enter the turbine intake and are

carried to increasing depths upon approaching the turbine runner. As the fish pass between the turbine runner blades, they are exposed to a sudden pressure decrease (<1 s) before returning to near surface pressure as they enter the downstream channel  $(\sim 20 \text{ s})$ . The magnitude of the pressure decrease during turbine passage is dependent upon the turbine runner design, the operation of the turbine, the submergence of the turbine runner (i.e., elevation of the turbine runner relative to the downstream water surface elevation), the total project head, and the flow path (Čada 1990; Carlson et al. 2008; Deng et al. 2010). The overall pressure change will increase with increased project head; the total project head (the difference between the upstream and downstream water surface elevations) is between approximately 17 and 30 m at lower Snake and Columbia River hydropower projects. Pressures are generally higher near the front side of the turbine blade (upstream pressure side) and lower near the back side of the turbine blade (downstream suction side), and they are typically (but not always) lower near the blade tips than in the midblade region (ENSR Corporation 2008). A turbine with a deep submergence will generally have higher nadir pressures than a turbine with a shallow submergence, and for any given turbine the nadir pressure will decrease with increased turbine flow. The lowest possible pressure experienced by a fish during turbine passage can vary from approximately -2 to 200 kPa (Carlson et al. 2008; Deng et al. 2010) depending on turbine design, operation, head, and passage route. However, nadir pressures lower than the median exposures used here for Kaplan turbines are probably less common than nadir pressures above the median exposures.

*Fish removal and necropsy.*—After STP exposure, fish were euthanized with an overdose of MS-222 (250 mg/L). Necropsies were performed as detailed by Stephenson et al. (2010). Mortal injury served as the endpoint of all analyses.

Mortal injury.—After the fish were exposed to STP, many fish died within a few minutes or received pressure-related injuries (barotraumas) that were sufficient to cause eventual mortality. It is not always feasible to hold fish after rapid decompression testing, and the conditions in which fish can be held are highly variable (pressure, temperature, total dissolved gas, or other conditions may vary). Therefore, instead of using mortality and a multitude of different injury types as response variables in this study, we used a metric for predicting mortal injury (derived by McKinstry et al. 2007) as the response variable. The mortal injury metric was derived by analysis of a large data set of fish exposed to rapid decompression. The metric associated the fish that died within minutes of rapid decompression with the injuries that were observed during necropsy. The injuries that were seen most often in fish that died were included in the metric (McKinstry et al. 2007).

The first step in building this metric was to make odds ratios from  $2 \times 2$  contingency tables that associated mortality with the presence or absence of each injury type. Fisher's exact test was used to determine whether an injury was significantly associated with mortality. The 28 injuries that were significantly associated with mortality were then entered into a stepwise logistic model under step 2 of the analysis. Model building is the process of selecting a best subset of predictor variables in a stepwise regression model from a larger set based on the Akaike's information criterion (AIC) value (Hosmer and Lemeshow 2000; Venables and Ripley 2000). The AIC is a goodness-of-fit measure of an estimated statistical model and assesses the trade-off between the goodness of fit and the number of variables included in the model. The injuries retained by this model included exophthalmia (pop-eye); hemorrhaging in the pericardium, liver, or kidney; ruptured swim bladder; blood or bile secretions from the vent; and emboli in the gills or pelvic fins. Step 3 of the analysis was to fit these eight injuries into another logistic regression model and test the significance of individually adding all 28 possible two-way interaction terms. No two-way interaction terms were found to be significant. Although the presence of emboli in the pelvic fins appears to be an injury that would not be associated with mortality, among the fish that exhibited this malady (n = 416), 340 (81.7%) died during or within a few minutes of exposure to rapid decompression (Table 3). Thus, the presence of emboli in the pelvic fins acted as an externally observable predictor of mortality. Therefore, mortal injury served as the endpoint and response variable for our analyses, and fish with any one of these eight injuries present or fish that were dead shortly after testing (within  $\sim 10$  min) were classified as mortally injured. Although we noted other injuries that could lead to delayed mortality or an increased chance of predation, they were not highly associated with mortality shortly after STP and therefore were excluded from this analysis.

*Statistics.*—The experimental factors examined in this study were (1) LRP (as detailed by Brown et al. 2012), (2) tag burden, (3) transmitter type, (4) condition factor (a measure of energy

TABLE 3. Number of juvenile Chinook salmon that had one of eight injuries that were highly correlated with mortality and the number and percentage of those fish that died soon after exposure to rapid decompression during simulated turbine passage. The percentage of all mortally injured fish (5,318 fish were mortally injured among the 10,742 study fish) that had a particular mortal injury is also presented; many fish had multiple mortal injuries.

Type of injury	N with injury	N that died	Percentage that died	Percentage of all mortally injured fish
Swim bladder rupture	5,077	231	4.5	95.5
Exophthalmia	226	130	57.5	4.2
Hemorrhaging				
In heart	690	364	52.8	13.0
In liver	722	120	16.6	13.6
In kidney	309	286	92.6	5.8
Blood in vent	276	24	8.7	5.2
Emboli				
In gills	1,290	173	13.4	24.3
In pelvic fin	416	340	81.7	7.8

storage [weight divided by the cube of length, multiplied by 10<sup>5</sup>]; Anderson and Neumann 1996), (5) fish FL, and (6) fish weight. In total, 11,375 fish received one of the five transmitter combinations and then were either exposed or not exposed to STP in a replicated experimental design. Of those fish, 10,742 were included in statistical analysis. The remaining fish were excluded because they did not achieve neutral buoyancy, expelled a transmitter, or died prior to exposure. Data from external examinations followed by necropsy for the presence of barotraumas (i.e., those that were identified as mortal injuries) were used in the subsequent analysis only if nadir pressures were 144.4 kPa or lower during STP.

Statistical models.—Mortal injury was modeled by using general linear models based on a logistic link function and Bernoulli error structure. Analysis of deviance was used in modeling the data and testing hypotheses, where

 $y_{ij} = \begin{cases} 1 \text{ if the fish died or was mortally injured} \\ 0 \text{ otherwise} \end{cases}$ 

Concepts of r or  $r^2$  are not very meaningful in binary (0, 1) regression, and an alternative expression of model performance was necessary. Logistic model performance was evaluated by the area-under-the-curve (AUC) method (Hosmer and Lemeshow 2000). For example, in randomly flipping a coin, one cannot expect to predict the correct outcome more than 50% of the time. One cannot deterministically predict a truly random event. Hence, there is a baseline level of performance below which one can never accurately model. The baseline is the lower diagonal on the AUC plot (Figure 1), which plots the truepositive rate versus the false-positive rate. A cutoff probability is selected above which the predicted probability is classified as a "success" and below which the probability is classified as a "failure." The predicted classification is then compared with the observed outcome to calculate the true-positive and false-positive rates. The AUC curve then plots the true-positive rate versus the



FIGURE 1. Example of an area-under-the-curve plot with alternative model performance curves.

TABLE 4. Sequential analysis of deviance of the factors associated with mortal injury of tagged juvenile Chinook salmon exposed to simulated turbine passage. The final model explained 41.9% of the variability in the data (see text for details; LRP =  $\log_e$  transformed ratio of acclimation pressure : exposure pressure; TB = tag burden; *L* = fish fork length, mm; TT = tag type; CF = condition factor). Factors to the right of the "!" symbol were already in the model when the factor to the left was examined.

			Mean		
Source	df	Deviance	deviance	F	Р
Total corrected	10,741	14,694.2			
Main effects					
LRP	1	5,237.2	5,237.2	5,947.8	< 0.0001
TB   LRP	1	930.3	930.3	1,171.7	< 0.0001
$L \mid LRP + TB$	1	51	51	64.6	< 0.0001
TT   LRP + TB + L	4	15.4	3.9	4.9	0.0006
CF   LRP + TB + L + TT	1	9.1	9.1	11.6	0.0007
Interactions					
$(L \times TT)   LRP + TB + L + TT + CF$	4	32.4	8.1	10.3	< 0.0001
$(L \times CF)   LRP + TB + L + TT + CF + (L \times TT)$	1	29.8	29.8	38.1	< 0.0001
$(LRP \times TB)   LRP + TB + L + TT + CF + (L \times TT) +$	1	20.7	20.7	26.5	< 0.0001
$(L \times CF)$					
$(LRP \times L)   LRP + TB + L + TT + CF + (L \times TT) + (L$	1	20.7	20.7	26.6	< 0.0001
$\times$ CF) + (LRP $\times$ TB)					
$(LRP \times TT)   LRP + TB + L + TT + CF + (L \times TT) +$	4	15	3.7	4.8	0.0007
$(L \times CF) + (LRP \times TB) + (LRP \times L)$					
$(TB \times TT)   LRP + TB + L + TT + CF + (L \times TT) + (L$	3	7.8	2.6	3.3	0.0195
$\times$ CF) + (LRP $\times$ TB) + (LRP $\times$ L) + (LRP $\times$ TT)					
$(LRP \times CF) \mid LRP + TB + L + TT + CF + (L \times TT) +$	1	4.2	4.2	5.3	0.0213
$(L \times CF) + (LRP \times TB) + (LRP \times L) + (LRP \times TT)$					
$+$ (TB $\times$ TT)					
Error	10,718	8,320.5	0.78		

false-positive rate for all possible values of the cutoff probability. We compared AUC values (AUC inside the unit square) between alternative logistic models to identify their relative levels of performance.

## RESULTS

#### Factors Associated with Mortal Injury

The main treatment factors of LRP (P < 0.0001), tag burden (P < 0.0001), fish FL (P < 0.0001), tag type (P = 0.0006), and condition factor (P = 0.0007) and several interaction effects were significant predictors of mortal injury for tagged juvenile Chinook salmon (Table 4). Fish weight was not a significant predictor (P = 0.2367). The main model plus the seven interactions explained 43.3% of the variability in the data. The LRP explained the most variability in the model (35.6%), and tag burden explained 6.3% of the variability. The other variables and interaction factors combined explained less than 1% of the variability in the model (fish FL, 0.3% of the variability; tag type, 0.1%; condition factor, 0.06%; fish FL × tag type, 0.2%; fish FL × condition factor, 0.07%; LRP × tag burden, 0.006%; LRP × fish FL, 0.006%; LRP × tag type, 0.1%; tag burden × tag type, 0.05%; LRP × condition factor, 0.03%).

Similar to the analysis of deviance, the AUC analysis also indicated that the LRP and tag burden were the most influential factors in determining the likelihood of mortal injury. The model containing all significant effects and interactions had an AUC score of 0.9055 (Figure 2). The model containing only LRP explained a large portion of the variability (AUC score = 0.8783). However, the model containing LRP and tag burden had an AUC score of 0.9034, thus explaining all significant variability. The simplified model that included LRP and tag burden appeared to be an adequate predictor of mortal injury.

Analysis of deviance of this simplified model indicated that LRP and tag burden were significant predictors of mortal injury (both P < 0.0001; Table 4), explaining 41.9% of the variability in the model. Equation (1) for predicting mortal injury given LRP and tag burden (%) is

Probability of mortal injury = 
$$\frac{e^{-5.997+4.201 \cdot \text{LRP}+0.603 \cdot \text{TB}}}{1 + e^{-5.997+4.201 \cdot \text{LRP}+0.603 \cdot \text{TB}}},$$
(1)

where TB = tag burden. Using the data collected in this study, three-dimensional plots were constructed to display the relationship between mortal injury and LRP or tag burden (Figure 3).



FIGURE 2. Area-under-the-curve (AUC) plots of the predictive value from each progressive model and the actual mortal injury outcome for juvenile Chinook salmon that were exposed to simulated turbine passage (LRP =  $\log_e$ transformed ratio of acclimation pressure : exposure pressure; TB = tag burden; L = fish fork length; TT = tag type; CF = condition factor).

These plots demonstrate that as LRP and tag burden increase, mortal injury also increases.

### **Fish Injuries and Mortality**

Overall, 5,318 (49.5%) of the 10,742 fish that were exposed to rapid decompression in this experiment had at least one mortal injury. Among fish with injuries that were highly associated with mortality, some injuries (e.g., hemorrhaging of the kidney: 92.6% likelihood of mortality) had a high probability of leading to death within a few minutes after rapid decompression (Table 3). The presence of emboli in the pelvic fins was an excellent external indicator of mortality due to barotrauma. Some injuries, such as a ruptured swim bladder (occurring in 95.5% of mortally injured fish), were very common among fish exposed to rapid decompression but typically did not lead to rapid mortality; only 4.5% of fish with ruptured swim bladders died within a few minutes of exposure. However, we noticed that many of the fish with ruptured swim bladders retained a large amount of gas within their body cavity after exposure and were positively buoyant.

## Tag Expulsion

Throughout the study, three acoustic tags (0.06%) and six PIT tags (0.12%) were expelled during STP exposure. Acoustic tags



FIGURE 3. Overlays of fitted logistic curves of mortal injury in relation to the  $\log_e$  transformed ratio of acclimation pressure : exposure pressure (ratio of pressure change) and the tag burden (tag mass expressed as a percentage of fish mass) for juvenile Chinook salmon exposed to simulated turbine passage. Plots were rotated on the *z*-axis of mortal injury to provide alternative perspectives of the surrounding plane. Tag burdens of 0.0–6.6% were tested in the present study.

were expelled over varying LRP values (one double-battery tag at LRP = 0.95; two single-battery tags at LRP = 2.22-2.74). Passive integrated transponder tags were also expelled over a wide range of LRPs (mean = 1.64; range = 1.38-2.43). Most (83.3%; 5 of 6) of the expelled PIT tags were from fish that had also received an acoustic tag; the remaining PIT tag was expelled from a fish belonging to the PIT-tag-only treatment group. Only a single fish lost both a PIT tag and an acoustic tag (single-battery acoustic tag + PIT tag treatment; LRP = 2.22). None of the fish that expelled tags died during or shortly after exposure to STP; however, 100% of fish (3 of 3) that expelled acoustic tags and 83.3% of fish (5 of 6) that expelled PIT tags were mortally injured as a result of STP exposure.

## DISCUSSION

#### Factors Associated with Mortal Injury

This research demonstrates that the derived variables—LRP and tag burden—are the most important factors in determining the likelihood of mortal injury for juvenile Chinook salmon that are exposed to rapid decompression associated with turbine passage. Because millions of juvenile salmon are tagged with telemetry tags (acoustic, radio, and inductive tags) each year to assess passage and survival through hydropower facilities, the implications of these results are important for understanding the effect of such facilities on salmonid survival during downstream migration. Bias in survival estimates of fish passing through hydropower facilities can lead to the use of inaccurate information for management of the affected species; based on the present findings, it is likely that results from many previous telemetry studies have been negatively biased.

Changes in pressure have previously been demonstrated as an important factor in predicting the likelihood of injury and mortality for juvenile Chinook salmon undergoing rapid decompression as a result of STP (Brown et al. 2009, 2012). However, Brown et al. (2009) examined the occurrence of injury and mortality for LRP values ranging from only 1.7 to 3.3 (ratio of pressure change = 5.3-27.0), while the present study examined the likelihood of mortal injury from no pressure change to an LRP of 3.12 (ratio of pressure change = 0.0-22.6). Only the current study and the study by Brown et al. (2009) have addressed the additional influence of a telemetry tag on barotrauma due to rapid decompression.

Our results indicate that as tag burden increases, the rate of mortal injury also increases. Other researchers (Brown et al. 2009) have also shown that the presence of a telemetry tag influences injury and mortality rates. Brown et al. (2009) examined the effects of rapid decompression associated with the presence of a telemetry tag (tag burden = 1.3-4.7%) for juvenile Chinook salmon. Smaller fish (subyearlings) acquired higher rates of injury and mortality associated with STP than larger individuals (yearlings). However, this could not be clearly linked to the tag burden carried by smaller fish because the tag burden was similar for both subyearlings and yearlings (subyearlings: mean tag

burden = 2.9%, range = 1.4-4.2%; yearlings: mean tag burden = 3.1%, range = 1.3-4.7%; Brown et al. 2009). The present work examined a broader range of tag burdens (0.0-6.6%) and looked specifically at the relationship between tag burden and the likelihood of mortal injury; furthermore, this study also expanded the relationship between tag burden and mortal injury over a wider range of LRPs. The highest rates of mortal injury were seen for fish that were exposed to high LRPs and subjected to high tag burdens. By comparison, fish that were exposed to low LRPs and low tag burdens exhibited lower rates of mortal injury. The understanding of this relationship is important for application to field studies examining fish survival through turbines at hydropower facilities.

Within the Columbia and Snake rivers, tag burden has varied considerably among field studies examining route-specific survival, including passage through hydroturbines. Fish have been tagged with a variety of tag types (including radio, acoustic, and PIT tags) that have varied in mass from 0.1 g (an implanted PIT tag; e.g., Achord et al. 2009) to 1.4 g or higher (combined weight of implanted radio tag plus PIT tag; e.g., Hockersmith et al. 2003). The size of fish used in studies of hydroturbine passage survival at U.S. Army Corps of Engineers (USACE) projects in the Snake and Columbia rivers also varies. The range of fish sizes used is difficult to discern, as many reports do not state the minimum and maximum sizes of study fish. Based on information available in reports, we estimated that tag burdens of the juvenile Chinook salmon used in these studies generally have ranged from 1.0% to 6.4% (although tag burdens for some studies were not reported) depending on the fish size and the tag type used (Table 5). Increased rates of injury and mortality resulting from tag burdens in these studies may have created bias in survival estimates.

To illustrate the interaction between tag burden and pressure change on mortal injury, we used equation (1) to determine the estimated probability of mortal injury for fish with a broad range of tag burdens representative of in-river studies (Figure 4). The difference in mortal injury among tag types is low when pressure change ratios are low. This is due to the relatively minimal occurrence of injury overall when pressure changes are nonexistent or slight. However, as the pressure change experienced by fish increases, the differences in mortal injury attributed to tag burden become more apparent, with higher mortal injury occurring among fish that have higher tag burdens. At very high pressure change ratios, there is little difference in mortal injury attributed to differences in tag burden. This is due to the fact that most fish, irrespective of tag burden, are mortally injured when they experience high ratios of pressure change. Thus, the bias in telemetry studies that examine mortality due to turbine passage will vary with both tag burden and pressure change exposure.

Based on the results of the current study, the tag burden of juvenile Chinook salmon used in survival studies (Table 5) may have influenced the reported turbine passage survival estimates. To illustrate the possible bias due to carrying a telemetry tag during turbine passage, we used the fish and tag sizes from a

Dam	Life stage	Tag types	Tag mass (g)	Median fish mass (g)	Median tag burden (%)	Survival estimate (%)	Reference
John Day	Subyearling	JSATS + PIT	0.525	20.0	2.63	72.8	Weiland et al. 2009
	Yearling	JSATS + PIT	0.585	40.0	1.46	85.5	Weiland et al. 2009
Bonneville	Subyearling	Acoustic + PIT	0.525	20.0	2.63	93.3	Faber et al. 2010
	Yearling	Acoustic + PIT	0.525	40.0	1.31	94.8	Faber et al. 2010
	Subyearling	JSATS + PIT	0.525	14.2	3.70	93.3	Ploskey et al. 2009
	Yearling	JSATS + PIT	0.585	34.7	1.69	94.8	Ploskey et al. 2009

TABLE 5. Summary of fish life stage, fish size, tag type, tag mass, and tag burden used in previous studies estimating the survival of juvenile Chinook salmon passing through hydroturbines in the lower Columbia River (JSATS = Juvenile Salmon Acoustic Telemetry System tag; PIT = passive integrated transponder tag). Studies that used gastrically implanted transmitters are excluded. Survival estimates are from the corresponding adjacent reference.

study by Weiland et al. (2009) as an example (Table 5). This example is deterministic in nature and only utilizes single values for critical variables. A more-realistic and more-accurate assessment would require a stochastic approach that uses the expected distributions of the variables. For a typical Chinook salmon subyearling (median mass = 20.0 g; median tag burden = 2.63%; Table 5) and yearling (median mass = 40.0 g; median tag burden = 1.46%; Table 5) that are acclimated to a depth of 3 m (130.9 kPa) and exposed to a nadir pressure of 60 kPa (LRP = 0.78; ratio of pressure change = 2.18), we would estimate the probability of mortal injury to be 24.3% and 13.7%, respectively (using equation 1). In contrast, an untagged subyearling and yearling under the same acclimation and exposure conditions would both have an estimated mortal injury probability of 6.2% (a decrease of 18.1% and 7.5%, respectively). We expect a high proportion of these mortally injured fish to die be-



FIGURE 4. Comparison of the probability of mortal injury associated with different tag burdens (TB;%) for juvenile Chinook salmon exposed to varying ratios of acclimation pressure : exposure pressure (ratio of pressure change) during simulated turbine passage. Tag burdens of 0.0–6.6% were tested in the present study.

fore reaching downstream telemetry arrays that assess survival; thus, survival rates of subyearling and yearling Chinook salmon will increase by up to 18.1% and 7.5%, respectively, for the discrete values of the variables selected for analysis. This expected change in survival can influence the overall survival rate for a hydropower facility depending on the proportion of fish passing through the turbines, which varies with species, time of year, and other factors. Facilities like those found in the lower Snake and lower Columbia rivers can have low turbine passage rates (<10%), whereas facilities without alternative passage routes for fish (i.e., those without juvenile bypass systems or spillways) can have turbine passage rates as high as 100%.

The examples above, which only provide a discrete case assessment of the risk of mortal injury for a population of turbinepassed fish, illustrate the importance of tag burden when estimating survival of turbine-passed fish. Some researchers have recommended that tag burden should be no higher than 2% of a fish's total mass (Winter 1996). This general "rule" has been questioned by several authors (Brown et al. 1999; Jepsen et al. 2002), who have found that some aspects of fish fitness (e.g., growth, survival, and swimming performance) are not influenced by higher tag burdens. However, the idea of minimizing the size of telemetry tags used in research continues to be suggested. The results of the current study support these ideas and indicate that more-accurate estimates of hydroturbine passage survival are likely to be attained by using the smallest possible telemetry tag. In addition to influencing fish survival through turbines, the presence of a negatively buoyant transmitter can also influence the buoyancy of fish and the depth at which they travel. Fish that are tagged with transmitters have been shown to compensate for the additional excess mass by adding volume to their swim bladders (Gallepp and Magnuson 1972; Fried et al. 1976; Perry et al. 2001), which will limit the range of depths in which they can achieve neutral buoyancy. Deng et al. (2012) designed two neutrally buoyant, externally attached acoustic transmitters that show promise for use in hydroturbine passage survival studies. These tags do not add any additional mass to the fish in water, thus eliminating the fish's need to compensate for the additional mass by adjusting swim bladder volume. The use of these tags for hydroturbine survival studies may be a

viable option for eliminating the bias we demonstrated as being associated with the presence of telemetry tags.

Several variables other than LRP and tag burden were statistically significant in the analysis of deviance model; however, they were not included in the final model due to their lack of predictive power. For example, several types of telemetry tags are employed by researchers to monitor fish behavior and survival. These tags encompass a variety of different shapes and sizes in addition to the differences in mass. Based on the results of this study, the type of tag used is important in determining the likelihood of mortal injury, but this importance is minute compared with that of the LRP and tag burden. Several researchers have examined the importance of tag shape among externally attached tags, as they have the potential to create drag while the fish is swimming and to snag on natural vegetation and debris that a fish may encounter (Ross and McCormick 1981; Winter 1996; Thorstad et al. 2001; Sutton and Benson 2003). Although this topic may be equally important, information on the influence of tag shape on fish that receive surgically implanted telemetry tags is lacking. For example, the shape of a tag may influence damage to internal organs when the swim bladder expands during rapid decompression, possibly pushing the tag into internal organs and thereby resulting in puncture and compression injuries. Although our study used telemetry tags of similar shapes, future research should focus on examining the influence of differences in tag shape on the likelihood of mortal injury for hydroturbine-passed fish.

Although it is likely that the volume occupied by a tag is highly correlated with the tag mass in currently used transmitters, the tag volume may also be an important variable in relation to barotrauma. During rapid decompression, the expansion of gases in the swim bladder and tissues will reduce the available volume (which is finite) within the intraperitoneal cavity where the tag rests. The presence of a tag may limit the volume to which gases can expand before barotraumas (e.g., compression-related injuries) occur; in fact, this may be the causal mechanism that explains the differential between tagged and untagged fish in the probability of mortal injury in response to rapid decompression. Objects that fill a portion of the body cavity would reduce the space available for normal swim bladder function and would limit the depth at which neutral buoyancy can be attained similar to the effect of additional mass. The increased risk of barotrauma and the reduced depth of neutral buoyancy would likely be additive as tag volume increases relative to tag mass. It is possible that two tags of equal mass but different volume could produce different barotrauma responses, particularly if the fish had to hold the same mass of gas in the swim bladder to achieve neutral buoyancy for both tags. Although the type of tag implanted was not included in the final model estimating the likelihood of mortal injury (due to low predictive power), it may be an important component and should be considered during future research.

The method of implantation may also influence barotrauma in hydroturbine-passed fish. Brown et al. (2009) reported that the likelihood of a juvenile Chinook salmon suffering injury or death as a result of STP was greater for fish that were gastrically tagged than for fish that were untagged or that received surgically implanted tags. It was hypothesized that this difference was influenced in part by the incision through the body wall of the fish; the incision was not fully healed during STP exposure and so may have promoted stretching of the body wall to permit the release of gases or increase the body cavity's volume, reducing the pressure on internal organs and thus possibly decreasing barotraumas. For the current study, all fish with implanted tags had some kind of vent to the intraperitoneal cavity (i.e., the surgical incision or the puncture from PIT tag insertion). Although mortal injury did not differ by tag type (PIT insertion versus surgical implantation) in the present study, the method of implantation (incision size variance due to tag size or type) may be an important consideration for understanding the influence of telemetry tags on hydroturbine-passed fish and thus is worthy of future research.

The condition factor and FL of fish were also statistically significant variables explaining the variability in observations of mortal injury; however, like tag type, they contributed very little to the predictions of mortal injury in comparison with LRP and tag burden. Fish weight was not a statistically significant factor explaining the variability in observations of mortal injury. However, fish weight was an element in the computation of condition factor and tag burden, both of which were statistically significant in explaining the variability in mortal injury.

## Fish Injuries and Mortality

Many of the injuries that we noticed in fish after rapid decompression did not lead to instantaneous mortality. Although some of them (e.g., hemorrhaging of the kidney) lead to quick death, others may lead to delayed mortality due to predation. Swim bladder rupture did not often result in rapid mortality, but since fish are typically left with buoyancy control issues and many are positively buoyant, delayed mortality or mortality due to predation is likely.

#### Tag Expulsion

Transmitter loss in fish used in survival studies can bias survival estimates because fish that lose transmitters cannot be detected and are functionally "dead." Thus, the minimization or elimination of transmitter expulsion is needed to ensure accurate interpretation of survival study results. Tag expulsion rates were very low among the fish tested in this study: only 0.06% of fish lost acoustic tags, and 0.12% of fish lost PIT tags. Brown et al. (2009) observed a radio tag expulsion rate of 3.1% (5 of 163 tags) among juvenile Chinook salmon that were gastrically tagged with radio transmitters and then exposed to STP; however, Brown et al. (2009) reported no expulsion among fish that received surgically implanted radio transmitters (tag burden = 1.3-4.7% for both implantation methods).

The number of sutures and type of knot used when incisions are closed could influence tag retention during hydroturbine passage. In the present research, two sutures were used to close each 6-mm incision (via methods described by Deters et al. 2010). Boyd et al. (2011) determined that when one suture was used instead of two sutures to close a 6-mm incision, the incidence of visceral expulsion through incisions after STP was higher. Thus, when examining fish survival through turbines, researchers should take care to close the incisions appropriately to prevent injury to fish and to prevent tag expulsion.

## **Conclusions and Recommendations**

Our study demonstrates that LRP and tag burden are the most important factors in estimating the likelihood of mortal injury for juvenile Chinook salmon that are exposed to rapid decompression associated with turbine passage. The results indicate that as tag burden increases, the rate of mortal injury also increases. Based on these results, the tag burden of juvenile Chinook salmon used in survival studies may have influenced the previously reported estimates of survival for turbine-passed fish. This, in turn, can influence overall dam passage survival estimates.

The results of this study have wide implications for the management of hydroelectric facilities. Our findings indicate that the presence of a telemetry tag creates a bias in estimated survival of juvenile Chinook salmon undergoing rapid decompression associated with hydroturbine passage. This is especially true for fish that undergo large changes from acclimation pressure to nadir pressure and for fish with large tag burdens. This knowledge has a direct impact on the interpretation of survival estimates for fish passing through hydropower facilities because past estimates may be biased toward higher rates of mortality than would have occurred for untagged fish. In the future, the smallest telemetry tag possible-both in volume and mass-should be used for turbine survival studies. In addition, research should be conducted to identify technology that can be used to determine accurate estimates of hydroturbine passage survival. One possible option for hydroturbine survival studies is the use of recently developed neutrally buoyant transmitters that are designed to be externally attached to juvenile salmonids (Deng et al. 2012). Previously reported turbine passage survival estimates based on telemetry should be used with some reservation in light of the potential negative bias from tag burden. Additional research should be conducted to understand the physiological state of fish prior to and immediately after turbine passage. Buoyancy has been outlined as an important factor in the rates of mortal injury; however, the buoyancy of fish entering hydroturbines is currently unknown. The expelling of gases from the swim bladder prior to turbine passage may decrease the severity of effects for turbine-passed fish. Studies in which tagged and untagged fish are captured immediately after turbine passage and are assessed for mortal injury should also be conducted. More research characterizing the pressures to which fish are exposed during turbine passage is needed.

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