Assessment of Barotrauma in Untagged and Tagged Juvenile Chinook Salmon Exposed to Simulated Hydro Turbine Passage

Final Report

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October 2010

Pacific Northwest
NATIONAL LABORATORY

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October 2010

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Summary

To obtain data needed to implement turbine survival testing at all dams within the Federal Columbia River Power System and provide information to aid design of replacement turbine runners, the U.S. Army Corps of Engineers (USACE), Portland District, contracted Pacific Northwest National Laboratory (PNNL) to assess the response of juvenile Chinook salmon (*Oncorhyncus tshawytscha*) to rapid decompression experienced during passage through Kaplan hydro turbines within dams on the mainstem Columbia River. PNNL fisheries scientists designed and conducted a study to identify those factors that influence the probability of mortal injury for untagged and tagged juvenile Chinook salmon.

The sequence of the work covered research from 2006 through early 2010. The research consisted of the development and testing of multiple hypotheses. These hypotheses and results directed the flow of work toward the findings and conclusions presented in this final report.

The research consisted of three main thrusts:

- designing, developing, and implementing a laboratory system for testing the influence of decompression on fish

- assessing factors that influence mortal injury to untagged juvenile Chinook salmon exposed to simulated hydro turbine passage

- assessing factors that influence mortal injury to tagged juvenile Chinook salmon exposed to simulated hydro turbine passage

The details of these three related efforts are documented in this report.

Chapter 1

In this chapter, we describe a novel system for testing the influence of decompression on fish and other aquatic animals. The Mobile Aquatic Barotrauma Laboratory (MABL) differs from previous systems in that the rates and range of pressure change, as well as levels of total dissolved gas, can be modified accurately and controlled accurately. Further, fish can be held for long periods with flow-through water, allowing them to become acclimated and neutrally buoyant to pressures and dissolved gases while not becoming hypoxic. We also present a case study that examines how the state of buoyancy (negatively vs. neutrally buoyant) at their migration depth is associated with damage to fish during rapid decompression scenarios that simulate turbine passage. We hypothesized that neutrally buoyant fish would sustain more injury than negatively buoyant fish.

This study demonstrated that the MABL is a useful system for testing barotrauma because it controls covariates and addresses the limitations in previous barotrauma research. The case study demonstrated the importance of buoyancy, especially for physostomous fish, when assessing barotrauma and resulting immediate mortality. Specifically, juvenile Chinook salmon that are neutrally buoyant are at a much greater risk of injury and mortality during turbine passage compared to negatively buoyant conspecifics. This finding has important implications for research aimed at estimating the survival and behavior of juvenile salmonids as they pass through hydroelectric turbines. Further, our research suggests that previous research on turbine passage, in which fish were not allowed to become neutrally buoyant, may

be biased and underestimate barotrauma occurrence (specifically for physostomous fish). It also indicates the necessity of developing methods that would cause fish to become negatively buoyant prior to turbine passage, potentially reducing physiological injury and mortality in these economically and ecologically important fish species.

Chapter 2

The investigation described in this chapter was to determine what factors contribute to the incidence of barotrauma and mortal injury in hatchery-reared juvenile Chinook salmon exposed to rapid decompression associated with hydro turbine passage. Specifically, we exposed these fish to varied acclimation pressures and subsequent exposure pressures to mimic turbine pressure time histories (ratio of pressure change). Additionally, we varied the abiotic factors (total dissolved gas, rate of pressure change) and biotic factors (condition factor, fish length, fish weight) that may contribute to the incidence of mortal injury associated with fish passing through hydro turbines. Finally, we compared the influence of simulated turbine passage on naturally migrating (seaward-migrating) with that on hatchery-reared juvenile Chinook salmon.

We determined that the main factor associated with mortal injury of juvenile Chinook salmon during simulated turbine passage was the ratio between acclimation and nadir pressures. Condition factor, total dissolved gas, and the rate of pressure change were found to only slightly increase the predictive power of equations relating probability of mortal injury to conditions of exposure or characteristics of test fish during simulated turbine passage. Although this research provides valuable information about the relationships between rapid decompression and barotrauma for juvenile Chinook salmon, further research is needed to determine how other species are influenced by rapid decompression associated with turbine passage. This research should assist engineers and fisheries managers in operating and improving the efficiency of hydroelectric facilities while minimizing mortality and injury of turbine-passed juvenile Chinook salmon. With these data, models can be built that might determine how much mortal injury is present at different turbine operations as the turbine runner pressure environment changes. Further, pressure data coupled with the mortal injury data should be useful to engineers and turbine manufacturers when aiming to design new turbines that could not only increase power generation and efficiency but also minimize barotrauma injuries as fish pass through more fish-friendly turbines.

Chapter 3

Each year, the USACE surgically implants millions of fish with telemetry tags (acoustic, radio, inductive) to assess their passage and survival through hydropower facilities. One route of passage of particular concern is through hydro turbines, in which fish may be exposed to a range of potential injuries, including barotraumas from rapid decompression. The change in pressure from acclimation to exposure (nadir) has been found to be an important factor in predicting the likelihood of mortal injury for juvenile Chinook salmon undergoing rapid decompression associated with simulated turbine passage. The presence of telemetry tags has also been shown to influence the likelihood of injury and mortality for juvenile Chinook salmon. Tagged fish must increase the volume of their swim bladder to compensate for the added tag mass and attain neutral buoyancy. The additional gas in the swim bladder in conjunction with the presence of the tag makes the fish more susceptible to injury during rapid decompression.

The study featured in this chapter investigated the likelihood of mortal injury for juvenile Chinook salmon carrying one of four acoustic and inductive telemetry tag combinations (double-battery acoustic tag and passive integrated transponder [PIT] tag, single-battery acoustic tag and PIT tag, single-battery acoustic tag only, and PIT tag only; tag burden percentage (mass of the tag(s) divided by the fish's mass and multiplied by 100): 0.0% to 6.6%) and exposed to a range of LRP values (natural log of the ratio between acclimation and nadir pressure; LRP: no pressure change to 3.12). Several factors were examined as predictors of mortal injury for fish undergoing rapid decompression, and LRP and tag burden were determined to be the most predictive factors. As LRP and tag burden increase, the likelihood of mortal injury also increases.

The results of this study suggest that previous survival estimates of juvenile Chinook salmon passing through hydro turbines may have been biased due to the presence of telemetry tags, and this has direct implications to the management of hydroelectric facilities. Realistic examples indicate how the bias in turbine passage survival estimates could be 20% or higher, depending on the mass of the implanted tags and the ratio of acclimation to exposure pressures. Bias would increase as the tag burden and pressure ratio increase, and bias has direct implications on concrete survival estimates. It is recommended that future survival studies use the smallest telemetry tags possible to minimize the potential bias that may be associated with carrying the tag. In addition, research should be conducted to identify technology that can be used to determine accurate estimates of hydro turbine passage survival.

Overall Conclusions and Recommendations

Given the USACE efforts to more appropriately address how juvenile Chinook salmon respond to pressure stressors found during turbine passage, the current study aimed to develop a system that would accurately and precisely expose depth-acclimated fish to simulated hydro turbine pressure profiles and subsequently quantify the incidence of mortal injury following these pressure exposures. Further, given the importance of using tagged juvenile salmonids, with specific emphasis on Juvenile Salmon Acoustic Telemetry System tagging technology, efforts were directed at understanding what, if any biases in response to rapid decompression occurred in tagged fish exposed to simulated hydro turbine pressure profiles. The combined results of these efforts support several overall conclusions.

The design and application of the Mobile Aquatic Barotrauma Laboratory highlights the importance of acclimating fish to depth (pressure) prior to exposing them to tests of simulated turbine passage to gain the most accurate data. In addition, juvenile Chinook salmon physiologically respond to ratios of pressure change in a predicable manner. Covariates to the primary response variable, mortal injury, such as total dissolved gas, rate of pressure change, and condition factor, were of little importance in improving our understanding of the probability of mortal injury as a function of acclimation to exposure pressure ratio. Finally, results of more than 10,000 pressure tests on juvenile Chinook salmon indicate that the presence of a tag within the body cavity increases a fish's likelihood of becoming mortally injured following turbine passage. As tag burden increases, so does the likelihood of mortal injury. These findings and observations represent the most comprehensive study of how physostomous fish with and without tag burden respond to rapid decompression during turbine passage.

Based on the research results presented in this report, our conclusions and recommendations for future research are as follows:

- The MABL was designed to simulate the complex pressure time histories most significant of which is the rapid decompression fish experience during turbine passage. This system has proven its utility for investigation of the effect of rapid decompression on turbine-passed fish and would be a useful tool with which to address the effects of rapid decompression associated with other bypass alternatives. In particular, research is needed to examine the influence of large impulsive changes in pressure to which fish are exposed when carried in flow under the tainter gates that control spill discharge at most mainstem Columbia River dams.

- In addition to pressure time history, the MABL can precisely control covariates that have been identified as factors that influence the condition of downstream migrating juvenile salmonids both in federal hydropower system dam reservoirs and fish passage routes through dams. These covariates include factors such as the rate of pressure change occurring during turbine passage, water temperature, and the total dissolved gas prior to, during, and following turbine passage. These variables may play important roles in future research of turbine-passed fish, especially when these variables are found to be outside the range that we tested in the current study (i.e., total dissolved gas levels higher than 125% or rate of change in pressure greater than 500 psi/s). Research should be conducted to examine the influence of barotrauma on fish when higher levels of total dissolved gas and greater rates of pressure change occur.

- We have shown that a fish's buoyancy state prior to turbine passage is a highly significant predictor of the probability of injury and mortality that may occur from exposure to rapid decompression during turbine passage. Currently, researchers must assume that in general, fish traveling downstream are neutrally buoyant. This assumption is made because the energetic costs of swimming when negatively buoyant would decrease a fish's chances of successfully migrating to sea, and therefore this trait would not be selected for in future generations. Research (either in the field or the laboratory) that assesses the condition or mortality of fish during rapid decompression should clarify whether fish were given the opportunity to become neutrally buoyant and whether they obtained neutral buoyancy prior to turbine passage or simulated passage (rapid decompression). We suggest that experiments that do not account for the state of a fish's buoyancy prior to treatment exposure may provide results that are biased and cannot be applied to run-of-river fish. The design of future studies should therefore provide test fish the opportunity to achieve neutral buoyancy and should report the state of buoyancy of test fish at treatment as required meta data.

- In studies of rapid decompression during turbine passage, the ratio of pressure change, rate of pressure change, total dissolved gas, and condition factor are important predictors of the probability of mortal injury for untagged fish. However, when all statistically significant predictor variables are modeled together, the ratio of pressure change is the single best predictor of mortal injury for untagged juvenile Chinook salmon.

- Fish size (fork length or mass) was not a statistically significant variable in estimating the probability of mortal injury for turbine passed untagged fish, over the range of subyearling to yearling fish sizes tested (71–184 mm and 3.7–61.2 g, respectively). Other physiological measures not tested in this study likely would be significant predictor (explanatory) variables and should be examined in future studies of rapid decompression.

- The ratio of tag weight to fish weight (tag burden) is a highly significant predictor variable for the probability of mortal injury for turbine-passed fish. Specifically, when a tagged fish is compared to an untagged fish, both of which are subjected to the same ratio of pressure change, the probability of mortal injury is greater for the tagged fish. The probability of mortal injury also increases with tag burden. It is clear that mortality estimates for tagged fish that experience rapid decompression are biased in proportion to the magnitude of the tag burden for test fish. Any application of mortality estimates obtained under such conditions should acknowledge the potential for possibility of bias. We recommend that researchers minimize tag burden of test fish by utilizing the smallest tag possible for turbine passage studies. Although it is theoretically possible to estimate the bias in mortality estimates resulting from tag burden, it is complicated by uncertainties. These uncertainties include both the acclimation and exposure pressures of test fish and the typical range in tag burden over the size range of fish representative of a run-of-river population of interest.

- Future research should be conducted to examine how external neutrally buoyant tags may reduce or eliminate the bias found in those fish bearing uncompensated excess mass. Addition of excess mass in the form of a negatively buoyant internal or external tag forces a fish to increase its displaced volume by increasing the mass of gas in their swim bladder relative to an untagged fish acclimated to the same depth. This increase in swim bladder volume, and thereby whole body volume, makes them more susceptible to mortal injury during rapid decompression. This is due, presumably, to the higher forces exerted on internal organs and other tissues by the larger volume of gas contained within the swim bladder as it responds to decreases in pressure within the constraints of the fixed whole body volume of the fish. Therefore, tagging fish using an external neutrally buoyant tag should greatly reduce or eliminate the increase in probability of mortal injury experienced by tagged fish exposed to rapid decompression.

- It is unclear how differences in behavior (e.g., swimming depth), morphology (presence of a swim bladder, physostomous vs. physoclistous), and physiology (sensitivities and stress responses) affect how different species respond to rapid decompression during turbine passage. Therefore, there is an ongoing need to examine how sensitive species in a particular environment respond to rapid decompression during turbine passage while considering these species' specific differences. For example, within the Columbia River basin, sockeye salmon are widely considered to be more sensitive to injury than Chinook salmon, given the same exposure. Thus, they may be a better reference species for use when managing or designing new hydro turbines, assuming the goal of new turbine designs is to improve passage conditions for species most susceptible to injury from rapid decompression and other exposures. In addition, juvenile lamprey may also be highly susceptible to barotrauma due to the high pressures to which they are acclimated, as they have been observed to migrate downstream at greater depths than salmonids. This would lead to large differences between acclimation and exposure pressures during turbine passage and increase the potential for barotrauma when gas in the blood and tissues come out of solution during rapid decompression. There is a need to understand the theoretical depth of neutral buoyancy and the distribution of depths of neutral buoyancy for juvenile salmon migrating downstream through the Columbia River federal hydropower system. Acclimation pressure (correlated to the depth of neutral buoyancy) is the numerator in the ratio of pressure change that has been determined to be the primary predictor of the probability of mortal injury for fish exposed to rapid decompression. We recommend field and laboratory studies be conducted to better understand this topic. Of special interest is investigation of whether or not tag

burden influences the depth distribution of tagged fish. We hypothesize, based on observations from our study, that the depth of tagged fish is biased toward a shallower depth distribution in proportion to the magnitude of tag burden.

- This research emphasizes the importance of the mass of gas in a juvenile Chinook salmon's swim bladder at exposure to rapid decompression in relation to the fish's susceptibility to mortal injury. During our study, we observed the tendency of juvenile salmon to burp gas from their swim bladder when startled. Devices or methods that could reduce the amount of gas in a fish's swim bladder prior to turbine passage could minimize the probability of mortal injury for turbine-passed fish. We recommend investigating means to cause juvenile salmon to burp gas from their swim bladder prior to turbine runner passage.

Acknowledgments

Abbreviations and Acronyms

AIC	Akaike's information criterion
ARL	Aquatic Research Laboratory
AUC	area under the curve
°C	degree(s) centigrade
CF	condition factor
cm	centimeter(s)
FL	fork length
ft	foot, feet
g	gram(s)
GUI	graphical user interface
h	hour(s)
in.	inch(es)
km	kilometer(s)
L	liter(s)
LRP	ratio of acclimation to exposure (nadir) pressures with natural log applied
m	meter(s)
MABL	Mobile Aquatic Barotrauma Laboratory
mg	milligram(s)
min	minute(s)
mm	millimeter(s)
mmHg	millimeters mercury
MS-222	tricaine methanesulfonate
N	population size
n	subsample size
P	probability
PIT	passive integrated transponder
PNNL	Pacific Northwest National Laboratory
psi	pounds per square inch
psia	pounds per square inch–atmospheric
ROC	rate of pressure change
s	second(s)
±SE	plus or minus standard error
STP	simulated turbine passage
TDG	total dissolved gas
USACE	U.S. Army Corps of Engineers

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Chapter 1

Assessing Barotrauma in Neutrally and Negatively Buoyant Juvenile Salmonids Exposed to Simulated Hydro Turbine Passage Using a Mobile Aquatic Barotrauma Laboratory

John R. Stephenson, Andrew J. Gingerich, Richard S. Brown,
Brett D. Pflugrath, Zhiqun Deng, Thomas J. Carlson, Adam G. Seaburg

Introduction

Injury in fish due to rapid decompression (barotrauma) has implications for many areas of fisheries ecology throughout the world. Barotrauma-related injuries are commonly associated with fisheries management practices and applications such as commercial and sport fishing, as well as hydroelectric project operation. Although research related to barotrauma is critical to managing fisheries, there is a paucity of research related to the area. Barotrauma occurs in fish when they are exposed to rapidly decreasing pressures. Gas in the swim bladders of fish expands during decompression and can lead to swim bladder rupture and compression-related injuries. In addition, as fish are decompressed, gas can come out of solution in the blood and tissues, leading to bubbles forming in the blood and tissues (emboli and emphysema) and rupturing of the vasculature or hemorrhaging (Cramer and Oligher 1964; Tsvetkov et al. 1972; Beyer et al. 1976; Rummer and Bennett 2005; Brown et al. 2009).

Injury due to barotrauma is common among fish passing through hydroelectric turbines (Cramer and Oligher 1964; Čada 1990, 2001; Brown et al. 2009). Particularly vulnerable fish include migratory species, such as anadromous salmonids (Čada 2001) and other anadromous non-salmonids, including river herring (alewife, *Alosa pseudoharengus*, and blueback herring, *A. aestivalis*; Eyler et al. 2002). Other examples include injury to fish that are transported by helicopter (Hauck 1986) or other aircraft when surrounding pressures decrease rapidly during flight. Barotrauma-related injury may also be seen among fish subjected to anthropomorphic underwater disturbances such as noise pollution from pile driving or seismic exploration (Carlson et al. 2008). Even eggs and other early life stages of fish may be vulnerable to the impacts of pressure change associated with pumped storage power plants and condenser cooling systems (Beck et al. 1975). Barotrauma also occurs when fish are captured and quickly brought to the surface during commercial fishing operations (Burns and Restrepo 2002; Radershausen et al. 2007). Recently, barotrauma has been a concern in catch-and-release angling (Rummer and Bennett 2005; Gravel and Cooke 2008; Wilde 2009), prompting many fisheries scientists to investigate management strategies that would address and minimize the negative effects caused by barotrauma.

Given the importance of barotrauma to fisheries ecology, there is a need to systematically test the variables responsible for the incidence of barotrauma and the resulting injury. Historically, field covariates (e.g., variation in water temperature, total dissolved gas, rate of pressure change) have prevented researchers from examining the specific pressure profiles of exposure. In the laboratory, the usefulness of simple hypo- (decreasing pressure) and hyperbaric (increasing pressure) chambers for conducting barotrauma research has been limited for several reasons. Some systems have been able to provide only static water scenarios; some have provided only constant or limited levels of total dissolved

gas, while others performed only simplistic pressure exposure profiles (e.g., Harvey 1963). Thus, experiments performed in these chambers may have limited biological or environmental relevance.

Many of these systems are as simple as a cylinder exposed to a vacuum pump, restricting the size and number of fish to be tested (e.g., Harvey 1963). However, Feathers and Knable (1983) used a larger hyperbaric chamber with more sophisticated controls, allowing for the holding of multiple fish for long periods while pressures were changed. Chamber size can limit the species, size, or quantity of fish that can be exposed to pressure variation. In addition, previous systems lack mobility, water conditioning, sterilization, and precise manipulation of a host of covariates (total dissolved gas, temperature, sensitive control of pressures over short periods [Abernethy et al. 2002]). Systems have been limited to the laboratory setting and have not been built to allow physostomous fish (such as salmonids) to become neutrally buoyant because they need to gulp air to fill their swim bladder, representing a key omission in barotrauma literature.

Knowledge of fish buoyancy prior to rapid decompression is crucial because air dissolved in tissues and contained within the swim bladder of fishes is responsible for pressure-related injuries. Therefore, injury due to rapid decompression should be related to the buoyancy state of fish. Researchers have found that damage to the swim bladder, internal hemorrhaging, and the occurrence of other injuries increased as pressure change increased (Rummer and Bennett 2005; Brown et al. 2009). To compensate for negative buoyancy (swimming head up) or positive buoyancy (swimming tail up), physostomous fish may adjust the mass of gas in the swim bladder by gulping at the water surface or expelling gas to attain neutral buoyancy. Therefore, a fish that is neutrally buoyant has more gas in its swim bladder than those that are negatively buoyant. Because the occurrence of swim bladder rupture increases with pressure change, the amount of gas in the swim bladder is related to damage, with more gas in the swim bladder leading to greater injury. Thus, the state of buoyancy prior to rapid decompression likely influences the injury incurred. However, most of the designs for hyper/hypobaric chambers have not allowed for fish to become acclimated. Consequently, these fish may not have achieved neutral buoyancy prior to testing while gas tensions in body fluids are more likely to be at equilibrium with those of the surrounding fluid.

In this chapter, we describe a novel system for testing the influence of decompression on fish and other aquatic animals. The Mobile Aquatic Barotrauma Laboratory (MABL) varies from previous systems in that fish can be held for long periods with flow-through water, the rates and range of pressure change are precisely controlled, and fish are allowed to become acclimated and neutrally buoyant to pressures and dissolved gasses while not becoming hypoxic. Further, the system allows for rapid decompression scenarios to be tested over a wide range of total dissolved gases. We also present a case study that examines how the state of buoyancy at depth (negative vs. neutrally buoyant) is associated with damage to fish during rapid decompression scenarios that simulate turbine passage. We hypothesized that neutrally buoyant fish would sustain more injury than negatively buoyant fish.

Methods

System Description

Each of the MABL's four chambers measures 45.7 cm inside diameter and 31.2 cm high, giving an interior volume of 51.2 L (Figure 1.1A). The chambers are made of 3.0-cm-thick acrylic glass and sealed at the top and bottom by 5.1-cm-thick aluminum. The chamber is surrounded by a Plexiglas lens that is

flat on all sides. The void between the lens and the chamber is filled with water, allowing non-distorted viewing. The chamber interior is accessed by a removable hatch, which covers a 30.5-cm-diameter opening. During operation, the testing area is enclosed by a vinyl curtain, providing isolation.
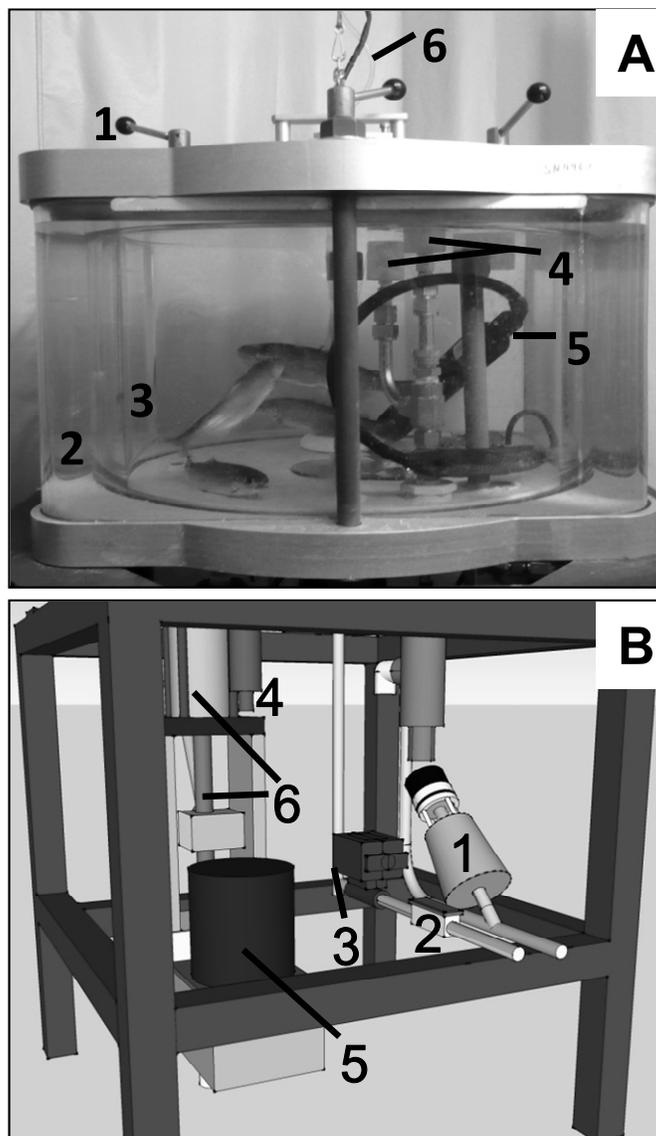
Figure 1.1. (A) One of the hypo/hyperbaric chambers of the MABL system. The chamber hatch is tightly closed with four dogs (1). A lens (2) surrounds the inner chamber (3) to allow for undistorted viewing. Float switches (4) maintain water level, and an in-chamber sensor (5) monitors total dissolved gas and temperature. Conditions are relayed by the system graphical user interface (not pictured). A bubble at the top of the chamber is maintained and removed by a release tube (6) during different phases of system operation, including acclimation and spike preparation. (B) The chamber sits on top of system controls consisting of an inflow control valve (1), vortex flow meter (2), temperature sensor (3), pressure sensor (4), servo motor (5), and a rod and piston (6) used to control rapid pressure changes. Note that panel B is a drawing to aid distinction of various components.

Water Flow and Pressure

Water flow through the chambers is controlled by a computer program with a graphical user interface (GUI; LabView, National Instruments Corporation, Austin, Texas). Flow-through water is delivered to chambers from an elevated head tank (125 L). The GUI adjusts the flow rate (±0.95 L/min. of target) allowing the chambers to hold acclimation pressure constant for greater than 72 h. Pressures can be maintained during the acclimation period between approximately 14.7 psia (surface pressure) and about 60 psia (105 ft depth). Because each chamber is controlled separately, the flow and acclimation pressures can be unique to each chamber simultaneously.

Water Quality

Fish may be exposed to elevated total dissolved gas (TDG) within impounded river systems. In the Columbia River, previous studies (Ebel and Raymond 1976; Weitkamp and Katz 1980) reported that elevated TDG of 115% to 143% had occurred, causing gas bubble disease and associated mortalities for juvenile and adult salmonids. In the MABL, a pack injection column (Point Four Systems Inc., Coquitlam, British Columbia) adds atmospheric gases up to 150% to water before it is transferred to the chambers. Sensors for TDG (Model T507, ±1.5 mmHg accuracy; In-Situ Inc., Fort Collins, Colorado) installed within each chamber are controlled by a separate computer program (written in CRBasic and implemented via LoggerNet) that provide real-time monitoring and save the measurements to a data logger (Model CR1000; Campbell Scientific, Logan, Utah).

Video Observation and Recording

Four color camcorders (Canon, Melville, New York) are operated at a rate of 30 frames/s and a resolution of 640 × 480 pixels to record general observations for extended time periods. The video camcorders are connected to a programmable digital video recorder (OpenEye Scientific Software, Inc., Spokane, Washington). Each chamber is equipped with computerized high-speed video systems (Redlake Digital Imaging Systems, Tucson, Arizona, or Basler Inc., Exton, Pennsylvania). These high-speed video systems are operated at 200 frames/s at a resolution of 640 × 480 pixels to capture fast behavioral responses during rapid decompression.

Experimental Procedures

Using the main computer program GUI, each chamber can be operated in four separate operational modes: Fill, Acclimate, Spike Preparation, and Drain. At any given time, each of the four chambers can be switched in and out of any of the four operational modes without disrupting the other chambers.

The Fill mode allows the chamber to be partially filled in preparation for loading fish (or other aquatic organisms) into the chamber. Following hatch closure, latches are securely dogged down (Figure 1.1A), and the Acclimation mode can then be initiated. The Acclimation mode delivers water into the chamber at a selected flow rate and acclimation pressure. This mode maintains an "automated air pocket" that allows physostomous species to gulp at the air space and become neutrally buoyant. The acclimation periods are necessary for physoclistous fish to achieve neutral buoyancy or aquatic organisms that do not have a swim bladder so that gases in tissues can equilibrate with surrounding pressures. To accommodate hyper/hypobaric conditions, the air pocket must be completely removed prior to

decompression (Figure 1.1A). The Spike Preparation mode prevents subtle pressure changes by slowly replacing the automated air pocket with water. After the air pocket is removed, a pressure change scenario (referred to in this chapter as a Spike) is initiated by directing the GUI to load the predetermined pressure simulation profile. This process activates a servo motor that controls a piston (Figure 1.1B) and subsequently initiates the pressure profile (Figure 1.1A). The GUI displays the complete exposure instantaneously and saves the actual pressure measurements sampled at 1,000 Hz. The Drain mode allows air to slowly enter while water is removed from the chamber to a depth of 2.54 cm without disrupting the surface pressure conditions. The chambers may be returned to the Acclimation mode if the fish are to be held for an extended post-treatment observation period at preselected pressure and flow conditions.

Case Study Description

Fish Acquisition and Handling

Both hatchery-reared and seaward-migrating juvenile Chinook salmon (*Oncorhynchus tshawytscha*) were exposed to simulated turbine passage (STP) treatments. Seaward-migrating yearling and subyearling Chinook salmon were collected daily between May 9, 2007, and August 22, 2007, from the Columbia River using the juvenile bypass facility at McNary Dam ($n = 599$). Sampled fish had a mean fork length of 112.8 mm (range, 80–165 mm) and a mean weight of 16.8 g (range, 6–58 g). Fish were held overnight, unfed, in a 125-L circular container with flow-through ambient river water prior to testing. Hatchery-reared yearling and subyearling Chinook salmon were either acquired as fry or hatched and reared from eggs at the Pacific Northwest National Laboratory (PNNL) Aquatic Research Laboratory (ARL). These fish were tested at the PNNL ARL between September 10, 2007, and May 30, 2008 ($n = 862$). Sampled fish had a mean fork length of 143.8 mm (range, 82–180 mm) and a mean weight of 34.1 g (range, 6.1–71.5 g). While at this location, the fish were held in 1,100-L circular holding tanks with flow-through ambient well water (mean temperature = 16.83°C ± 0.10 SE) and fed Biodiet moist pellets (Bio-Oregon, Longview, Washington) ad libitum. The same water was supplied to the MABL during this testing period.

For research conducted at McNary Dam, ambient river water (mean = 20.10°C, ± 0.24 SE) was supplied to the MABL via the juvenile passage facility. Fish tested both at McNary Dam and at the ARL were exposed to TDG of mean = 116.80% (±0.38 SE) and 115.51% (±0.25 SE), respectively. We elevated and maintained the TDG using the pack injection column described above. Treated water was supplied to all chambers at a continuous rate of 7.6 L/min with a flow control accuracy of ±0.95 L/min.

Before the fish were loaded into the chambers, they were individually netted from the holding tank and held in a bucket containing about 15 L of aerated water. Fish were then placed in a bath of tricaine methanesulfonate (MS-222; 80 mg/L of water) and PolyAqua (0.15 mL/L of water; Kordon Aquarium Products, Hayward, California) until they reached stage 4 anesthesia (Summerfelt and Smith 1990). While the fish were under anesthesia, fork length (FL; millimeters) and mass (grams) were measured. A small portion of the caudal fin was also removed while under anesthesia, giving each fish a unique marking for identification during the testing procedure. The fish were allowed to recover in four oxygenated 5-L buckets in groups of seven fish each while they achieved equilibrium. The number of fish was limited to seven per chamber to ensure the fish could be visually observed individually during the acclimation and STP exposure periods.

Loading Fish into the Chambers

Each bucket of fish was introduced into a partially filled chamber, and the GUI controls were set to the Acclimation mode, allowing the chamber to fill at a rate of 7.6 L/min and maintain an air pocket. Fish were given at least 16 h to acclimate to pressure equivalents of 25 ft (25.5 psia). During this acclimation period, fish were observed via video actively gulping air at the air pocket within the chamber, filling their swim bladders in an attempt to achieve neutral buoyancy. Following the acclimation period, researchers determined the state of buoyancy of each fish, which served as a predictor variable in our analyses.

Determination of Buoyancy

Using the video imaging system, we determined each fish to be negatively, positively, or neutrally buoyant. According to Harvey (1963), negatively buoyant fish tend to swim head-up/tail-down to remain off the bottom of the chamber, and they also exhibit elevated tail beat rates. Neutrally buoyant fish in Harvey's study were able to maintain a horizontal position within the chamber with minimal fin movement. Symptoms of positive buoyancy were exhibited by fish continuously struggling to move downward in the water column (head down/tail up) (Harvey 1963).

Simulated Turbine Passage Exposure

The original pressure data were collected using an autonomous Sensor Fish device (Deng et al. 2007). During the exposure, the chamber pressure was increased to approximately 58 psia over 20 s to simulate fish passing through a hydroelectric turbine intake and approaching the turbine runner (Figure 1.2). Brown et al. (2009) suggest that the compression of air-filled structures or dissolved gases within tissues are generally not subject to barotrauma. The fish were then subjected to rapid decompression within 0.5–3.5 s to nadir values (the lowest pressure) ranging from 1.8 to 18.6 psia, simulating passage through the suction side of a runner blade in the runner region. Finally, the GUI controlled the chamber pressure to simulate movement of fish out of the turbine draft tube and to the tailrace water surface when pressure returned to atmospheric (approximately 14.7 psia). The total STP exposure from the pressure increase in the intake to atmospheric pressure in the tailrace lasted approximately 40 s. The actual Spike pressure measurements were very close to the targeted pressure profile (Figure 1.2).

Fish Removal and Necropsy

At the conclusion of the STP exposure, any fish that died during the STP process were identified and noted. The chambers were then drained, and the fish were euthanized with an overdose of MS-222 (200 mg/L). Necropsies on all fish were performed within about 15 min to establish the presence or absence of gill emboli, swim bladder rupture, and presence of hemorrhaging in the liver, heart, or kidney. The body cavity of each fish was opened using rounded Bonn Artery Scissors (Fine Science Tools, Foster City, California) so that examiners would not puncture the swim bladder or other organs.

Statistics

Sequential analysis of deviance based on a logistic link function with a Bernoulli error structure was used to find the independent variables that produced the best-fit model for each binary response variable (Kutner et al. 2005). This type of model falls under the umbrella of generalized linear models. Wald

chi-square statistics and Akaike's information criterion (AIC) values were used to determine best-fit models for each binary response variable. The process of model building began by finding the best single covariate model, based on the Wald chi-square statistics and AIC values, then testing all remaining covariates together with the best single covariate and again testing the model fit. This process continued until no further covariates significantly improved the fit of the model. The presence or absence of a specific injury or mortality served as the binary response variables. Nadir, fish length, and fish weight served as continuous covariates, and state of buoyancy before pressure spike served as a binary covariate. Significance was assessed at $\alpha < 0.05$, and computations were performed using the computing program R (Version 2.9.1; The R Foundation for Statistical Computing).
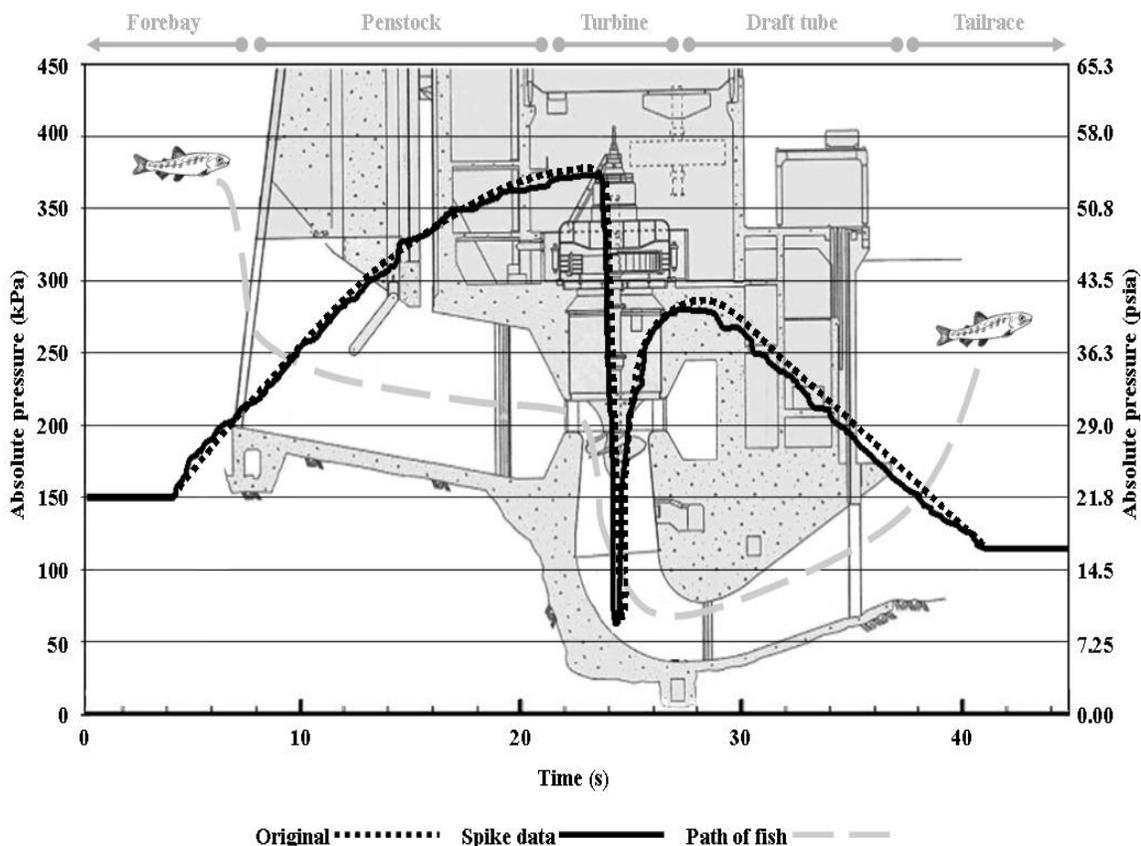
Figure 1.2. An example of the simulated turbine passage pressure profile and the spike pressure file measured inside the chamber by the MABL (black dotted and solid line), modified from Brown et al. (2009). The grey dashed line represents a typical path of a fish (pressure equivalents that the fish would experience are represented by the previously indicated black lines). The lowest pressure to which a fish is subjected within the turbine is termed the "nadir" and is illustrated at about 25 s on the *x*-axis.

Results

As nadir decreased, the incidence of immediate mortality increased, but it was much less common among negatively buoyant fish than neutrally buoyant fish (Figure 1.3a). Nadir ($P < 0.01$) and buoyancy ($P < 0.01$) were significant predictors of immediate mortality in juvenile Chinook salmon (Table 1.1). Neither weight ($P = 0.23$) nor length ($P = 0.14$) predicted mortality among the size range of fish we

tested. The main model explained 21% of the variability in the model; nadir explained 15%, and buoyancy explained 6%. The nonlinear logistic regression for the probability of mortality for a given nadir and buoyancy is presented in Equation (1.1), and the coefficients derived by the model are summarized in Table 1.2.

$$P_{\text{mortality}} = e^{-1.132-0.450*nadir+2.400*buoyancy}/1+e^{-1.132-0.450*nadir+2.400*buoyancy} \tag{1.1}$$
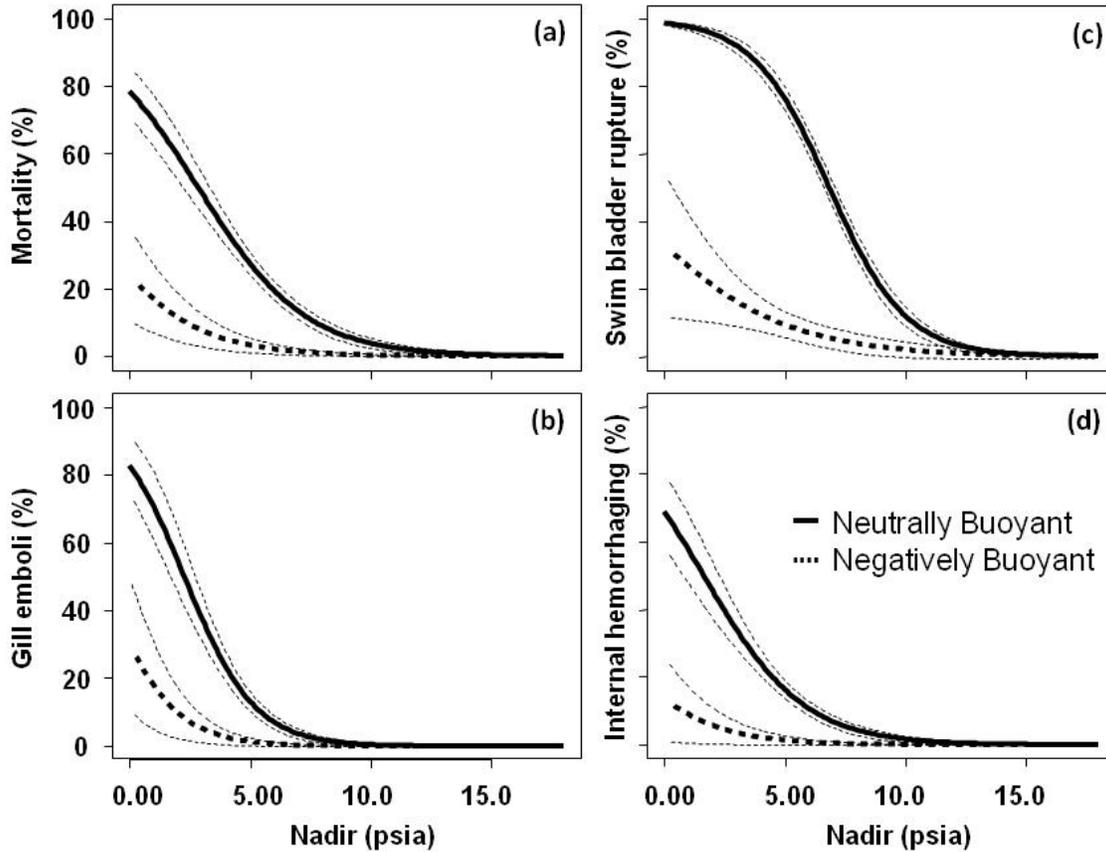
Figure 1.3. Resulting percentages of (a) emboli in the gills, (b) swim bladder rupture, (c) internal hemorrhaging (heart, liver, or kidney), and (d) mortality in fish that attained neutral buoyancy after acclimation and fish that did not achieve neutral buoyancy, at a given nadir. Standard deviations are expressed as small hashed lines about the dotted (negative buoyancy) and solid lines (neutral buoyancy).

Table 1.1. The analysis of deviance for the final models indicates that the main effects for nadir and buoyancy following simulated hydro turbine passage using the MABL system. Mortality, swim bladder rupture, gill emboli, and internal hemorrhaging served as dependent response variables. Factors to the right of the "|" symbol were already in the model when the factor to the left of the symbol was examined.

Response	Source	df	Deviance	Wald Chi-sq	P	AIC
Mortality	Total$_{cor}$	1460	1248.43			
	Nadir	1	186.06	$X^2 = 186.06$	< 0.01	1066.4
	Buoyancy	Nadir	1	79.06	$X^2 = 79.06$	< 0.01	989.3
	Error	1458	983.32			
Gill embolism	Total$_{cor}$	1460	913.79			
	Nadir	1	214.64	$X^2 = 214.64$	< 0.01	703.2
	Buoyancy	Nadir	1	55.25	$X^2 = 55.25$	< 0.01	649.9
	Length	Nadir + Buoyancy	1	33.25	$X^2 = 33.25$	< 0.01	618.6
	Error	1456	607.64			
Swim bladder rupture	Total$_{cor}$	1460	1962.92			
	Nadir	1	439.69	$X^2 = 439.69$	< 0.01	1527.2
	Buoyancy	Nadir	1	300.64	$X^2 = 300.64$	< 0.01	1228.6
	Nadir × Buoyancy	Nadir + Buoyancy	1	8.58	$X^2 = 8.85$	< 0.01	1222.0
	Error	1457	1214.01			
Internal hemorrhage	Total$_{cor}$	1460	909.26			
	Nadir	1	135	$X^2 = 135.00$	< 0.01	778.3
	Buoyancy	Nadir	1	54.65	$X^2 = 54.65$	< 0.01	725.6
	Error	1458	719.62			

As nadir decreased, the incidence of emboli in the gills increased. However, gill emboli were observed much less frequently among negatively buoyant fish and were seldom observed unless fish were exposed to nadirs less than approximately 5 psia (Figure 1.3b). Nadir ($P < 0.01$), buoyancy ($P < 0.01$), and length ($P < 0.01$) were significant predictors of the presence of emboli in the gills of juvenile Chinook salmon (Table 1.1). Weight was not a significant ($P = 0.61$) variable. The main model explained 33% of the variability in the model; nadir explained 23%, buoyancy explained 6%, and length explained 4%. The nonlinear logistic regression for the probability of emboli in the gill for a given nadir, buoyancy, and length is presented as Equation (1.2), and the coefficients derived by the model are summarized in Table 1.2.

$$P_{\text{Gill embolism}} = e^{2.829-0.691*nadir+2.413*buoyancy-0.028*length}/1+e^{-2.829-0.691*nadir+2.413*buoyancy-0.028*length} \tag{1.2}$$

Table 1.2. Coefficients for the models as defined by the analysis of deviance. The coefficient estimates divided by their standard error have an asymptotic normal distribution. Coefficients can be used in the response variable specific (mortality, swim bladder rupture, gill emboli, internal hemorrhaging) equation to predict the probability of occurrence at a given buoyancy and nadir following simulated hydro turbine passage. The lines generated from the equations are expressed in Figure 1.3.

Response	Coefficient	Estimate	SE	z	P
	Intercept	-1.13	0.38	-3.01	0.0026
Mortality	Nadir	-0.45	0.04	11.68	< 0.0001
	Buoyancy	2.40	0.36	6.69	< 0.0001
	Intercept	2.83	0.79	3.59	0.0003
Gill embolism	Nadir	-0.69	0.06	10.89	<0.0001
	Buoyancy	2.41	0.44	5.44	< 0.0001
	Intercept	-0.70	0.49	-1.44	0.1494
Swim bladder	Nadir	-0.31	0.09	-3.37	0.0008
rupture	Buoyancy	5.03	0.55	9.08	<0.0001
	Nadir × Buoyancy	-0.33	0.10	-3.27	0.0011
	Intercept	-1.85	0.54	-3.42	0.0006
Internal	Nadir	-0.49	0.05	-9.83	< 0.0001
hemorrhage	Buoyancy	2.64	0.52	5.07	< 0.0001

As nadir decreased, the incidence of swim bladder rupture increased and swim bladder rupture occurred much less often among negatively buoyant fish (Figure 1.3c). Nadir ($P < 0.01$), buoyancy ($P < 0.01$), and the interaction between nadir and buoyancy ($P < 0.01$) were significant predictors of swim bladder rupture in juvenile Chinook salmon (Table 1.1). The main model explained 38% of the variability in the model. Nadir (22%) and buoyancy (15%) explained most of the variability in the model, while the interaction between nadir and buoyancy explained little (0.4%). The nonlinear logistic regression for the probability of swim bladder rupture for a given nadir and buoyancy is presented in Equation (1.3), and the coefficients derived by the model are summarized in Table 1.2.

$$P_{\text{SB rupture}} = e^{-0.700-0.309*\text{nadir}+5.033*\text{buoyancy}-0.325*\text{nadir}*\text{buoyancy}} / 1 + e^{-0.700-0.309*\text{nadir}+5.033*\text{buoyancy}-0.325*\text{nadir}*\text{buoyancy}} \quad (1.3)$$

As nadir decreased, the incidence of hemorrhage increased, but it was seen less often in negatively buoyant fish compared to neutrally buoyant fish (Fig 1.3d). Nadir ($P < 0.01$) and buoyancy ($P < 0.01$) were significant predictors of hemorrhaging of the liver, heart, or kidney in juvenile Chinook salmon (Table 1.1). Neither weight ($P = 0.09$) nor length ($P = 0.13$) predicted mortality among the size range of fish we tested. The main model explained 21% of the variability; nadir explained 15%, and buoyancy explained 6%. The nonlinear logistic regression for the probability of internal hemorrhage for a given nadir and buoyancy is presented in Equation (1.4), and the coefficients derived by the model are summarized in Table 1.2.

$$P_{\text{hemorrhage}} = e^{-1.849-0.485*\text{nadir}+2.641*\text{buoyancy}} / 1 + e^{-1.849-0.485*\text{nadir}+2.641*\text{buoyancy}} \quad (1.4)$$

Discussion

Mobile Aquatic Barotrauma Laboratory Attributes

Results from the case study indicate that the MABL is a useful tool for examining barotrauma in aquatic organisms. The MABL provided a platform for conducting experiments while controlling acclimation pressures, total dissolved gas, and temperature. Unlike previous systems, it allowed physostomous fish to become neutrally buoyant before testing. In addition, it allowed for accurate replication of complex pressure change scenarios that replicate passage through a hydro turbine and enabled remote monitoring and recording of data variables.

Although we tested a narrow range of abiotic conditions (e.g., total dissolved gas, water temperature, pressure change), the MABL allows researchers to replicate actual decompression scenarios, which may have different constraints found in different applications. For example, the MABL could replicate pressures that a fish would encounter when caught commercially and discarded. Many different taxa could be tested in the MABL chambers. Furthermore, the MABL improves on previous pressure devices to control many covariates not considered in other barotrauma literature (e.g., dynamic flow-through conditions) while maintaining designated pressure; allows fish to acclimate for long periods; and allows fish to be tested at a wide range of total dissolved gases and temperatures.

Acclimation periods are essential for examining barotrauma in rapidly decompressed fish, as we have shown in this case study. Computer-programmed controls monitor and make micro adjustments to chamber conditions so that abiotic variables are accurately and precisely regulated. The computer controls also monitor and record real-time conditions in a data log while the user is absent so that this information may be reviewed and analyzed later, allowing the user to confidently collect and analyze predictor variables (e.g., nadir, acclimation pressure, total dissolved gas).

The MABL's mobility allows site flexibility. Previously, field testing at remote locations was not possible. However, a system such as the MABL can be moved to dams or other locations to evaluate the unique fauna in fishery systems, using ambient water. Mobility also potentially minimizes physiological changes that might be associated with transporting fish from the field to the laboratory. Further, the MABL's water conditioning equipment is capable of filtration, ultraviolet sterilization, temperature control, and total dissolved gas management to control for these covariates. Although fish were removed immediately following STP, the MABL allows for chambers to be refilled and acclimated to desired pressure so that researchers can quantify delayed mortality.

The computer program controls each chamber separately, allowing independent chamber operation. The system also allows remote data collection and delayed data analysis using readily available software products. Together, these attributes make the MABL an effective barotrauma testing facility that addresses many preexisting limitations of other decompression systems.

Importance of Buoyancy

The current case study illustrated that barotrauma and immediate mortality are dependent on fish buoyancy. Specifically, juvenile Chinook salmon that are neutrally buoyant are at a much greater risk of injury and mortality during turbine passage compared to negatively buoyant conspecifics. This finding

has important implications for research aimed at estimating the survival and behavior of juvenile salmonids as they pass through hydroelectric turbines. For example, our research suggests that previous laboratory research on turbine passage, in which fish were not allowed to become neutrally buoyant, may be biased and may underestimate barotrauma occurrence (specifically for those physostomous examples). For example, direct mechanical injury and mortality for turbine passage at hydroelectric dams are commonly tested using fish that are externally tagged with a "balloon tag" (Heisey et al. 1992; Mathur et al. 1996). These fish are released into turbines via a pipe induction system. The balloon tag is injected with a liquid prior to induction, causing the balloon to fill with air and the fish to float to the surface following passage. The fish are retrieved downstream of the dam with the aid of an externally attached radio transmitter. However, prior to being injected into the turbine, the fish are held on the top of the dam and handled in air prior to injection and thus are not acclimated to water depth as a natural seaward-migrating salmonid would be. As a result, fish have less air in their swim bladders prior to turbine passage. Our results indicate that tests evaluating passage without depth acclimation would likely result in a much lower injury estimate, thus potentially biasing mortality estimates.

Neutral buoyancy is a delicate balance of opposing forces (i.e., gravity and buoyancy) that minimizes the energy required to maintain a preferred location in the water column (D'Aoust 1973; Lefrancois et al. 2001). When the mass of the water displaced by a fish equals the fish's mass, the fish becomes neutrally buoyant. Brown et al. (2009) illustrated that fish acclimated to higher absolute pressures may be at increased risk of mortal injury because they experience a change of pressure of a higher ratio (acclimation pressure vs. exposure pressure), alluding to the importance of an extended acclimation period. Although, the ratio of pressure change is important in predicting injury or mortality (as detailed by Brown et al. 2009), all fish in the current study were acclimated to the same pressure (the pressure present at 25 ft, which is 25.5 psia). Therefore, exposure pressure (nadir) was used as an appropriate predictor of injury and mortality. Combined, this illustrates the need for research examining the maximum depth at which juvenile salmonids of various species can attain neutral buoyancy and a better understanding of this relationship in wild systems.

The results from this study lead to a possible anthropogenic means to reduce injury and mortality to fish passing through turbines. It has been well documented that some physostomus fish (such as salmonids) expel gas through their pneumatic duct when they become startled. Harvey (1968) noted that startled kokanee and sockeye salmon (*O. nerka*) tend to expel gas through their pneumatic duct and dive when they are startled. Expulsion of gas has also been observed during the current study in instances where fish were startled. This reduction of air in the swim bladder would cause fish to become negatively buoyant. We illustrated in this chapter that the probability of a negatively buoyant fish incurring severe injuries and mortality was drastically lower when compared to a neutrally buoyant fish when exposed to the same nadir. We suggest research aimed at provoking fish to expel gas before entering turbines, or other passage routes where rapid decompression may occur. This goal may be achieved through the use of sound, light, or other stimuli. Collectively, our research highlights the potential benefits of a device that would cause fish to expel gas in their swim bladders prior to turbine passage.

Many questions surrounding rapid decompression remain, which include delayed mortality and differences in injuries due to taxa-specific morphology and physiology. In this case study using the MABL, juvenile Chinook salmon were tested. Mortality and injuries were more common among neutrally buoyant fish. However, there was less difference between negatively and neutrally buoyant salmon in regard to internal hemorrhaging than immediate mortality and the other injuries we examined. Hemorrhaging of major organs is likely to cause delayed mortality. Furthermore, swim bladder rupture

likely makes fish susceptible to delayed mortality through predation because fish will likely not be able to regulate buoyancy. Future research should examine the importance of injuries attained during turbine passage on delayed mortality. Together, our estimates of instantaneous mortality should be considered a conservative representation of total mortality that can occur among turbine-passed juvenile salmonids.

It is difficult to predict how our results would align with physoclistous fish, in which no direct duct connects the swim bladder to the esophagus. Physoclistous fish equilibrate tissues and the swim bladder through circulatory system transfer (see Parker et al. 2006). Recommended research would allow for a predetermined acclimation period to eliminate disparity between acclimated and nonacclimated fish. Injuries to physoclistous fish are likely to occur at more dramatic rates at similar nadirs due to dissimilarities in morphological and physiological differences (e.g., direct connection found in the esophagus to the swim bladder in physostomous fish, allowing for air to exit more rapidly). Finally, there is a need to test fish without a swim bladder, such as the Pacific lamprey (*Entosphenus tridentatus*). Barotrauma studies on these species are underrepresented in this field of research. The assumption that these fish are not susceptible to mortality and barotrauma might be inaccurate because air dissolves in tissues and gas solubility increases with pressure at greater depths. Therefore, these types of fish may be susceptible to hemorrhaging and delayed mortality with acclimation to nadir pressure concerns similar to those of fish with a swim bladder.

Our case study was conducted within a very limited range of possible parameters related to environment and fish, to provide an example of the importance played in barotrauma research by the fish's state of buoyancy. Our results also illustrate the need for a system such as the MABL for conducting barotrauma research. Although we focused on only a few barotrauma-related injuries known to lead to mortality, further research is required to examine a broader spectrum of injuries that may result in immediate or delayed mortality. In addition, the effect of barotrauma injury and mortality over a range of varying ratios of acclimation to exposure pressures, as well as rates of pressure change present that fish would likely experience when passing through turbines, should be explored. A system such as the MABL should be used also to determine how a broad range of total dissolved gas present during acclimation and subsequent turbine passage may influence barotraumas. These data would be valuable for the management of existing hydro turbines and the design of new units.

Primary concerns in this field of research should include determining the maximum depth at which fish can attain neutral buoyancy and eliminating biases in survival studies through hydro turbines where tagged fish are used to represent the population. Emphasis needs to include decompression research on non-physostomous fish, including Pacific lamprey and even recreationally significant game fish species that are physoclistous. The use of systems like the MABL has the potential to assist turbine designers in developing new fish-friendly turbine designs, minimizing the impact on ecologically sensitive fish species.

Conclusion

This study demonstrates that the MABL is a useful system to test barotrauma because it controls covariates and addresses the limitations in previous barotrauma research. The case study demonstrated the importance of buoyancy, especially for physostomous fish, when assessing barotrauma and resulting immediate mortality. The results of this work support our recommendation to develop methods that

would provoke fish to become negatively buoyant prior to rapid decompression, potentially reducing physiological injury and mortality in these economically and ecologically important fish species.

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Chapter 2

Quantifying Mortal Injury of Juvenile Chinook Salmon Exposed to Simulated Hydro Turbine Passage

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Introduction

The survival of juvenile salmonids during turbine passage at hydroelectric projects continues to be an important environmental issue (Čada 2001; Brown et al. 2009). Mortality can be caused by fish colliding with turbine structure (e.g., contact with stay vanes, wicket gates, turbine runner), shear (forces applied parallel to the fish's surface resulting from two bodies of water of different velocities), grinding (passing through small gaps found between fixed and moving structures), turbulence, or changes in pressure (Čada 2001). Mortality associated with shear, grinding, or turbulence is often low among turbine-passed fish (Neitzel et al. 2000). However, all turbine-passed fish are exposed to changes in pressure. Therefore, pressure changes during turbine passage might be the most important variable associated with survival of salmonids traveling through hydroelectric projects.

Several researchers have determined that rapid decompression can lead to injury and mortality (i.e., barotrauma), and that the likelihood or quantity of damage that a fish incurs is related to both the pressure to which fish are acclimated and the pressure to which fish are exposed during turbine passage (Cramer and Oligher 1964; Beyer et al 1976; Brown et al. 2009). Depth-acclimated fish are neutrally buoyant. The fish body volume, which is a function of internal swim bladder pressure (and volume), displaces a mass of water equal to the fish's mass (Brown et al. 2009). Being neutrally buoyant may enable fish to conserve energy and maintain a preferred depth (D'Aoust 1973; Lefrançois et al. 2001).

The method of acclimation to depths below the water surface depends on whether fish are physoclistous or physostomous. Physoclistous fish, or those without a connection between the swim bladder and gut, move gas from their blood, filling their swim bladders using structures associated with the circulatory system (Moyle and Cech 1988). In physostomous fish, including juvenile Chinook salmon, a duct in their esophagus connects directly to their swim bladder, allowing them to gulp air at the water surface to fill their swim bladder. Although it is known that physoclistous fish become neutrally buoyant at a great range of depths (Shasteen and Sheehan 1997), it is unclear to what depths physostomes such as juvenile salmonids can attain neutral buoyancy (Brown et al. 2009).

For both physoclistous and physostomous fish, the depth of acclimation prior to decompression relative to the lowest exposure pressure (nadir) influences the magnitude of barotrauma and, ultimately, mortality (Beyer et al. 1976; Čada 1990; Abernethy et al. 2001, 2002, 2003; Rummer and Bennett 2005; Brown et al. 2009). For example, Rummer and Bennett (2005) and Brown et al. (2009) found that injuries and mortalities were most numerous and severe in fish subjected to the highest acclimation pressure relative to exposure pressure, or nadir (the lowest pressures to which fish are exposed).

Rapid decompression occurs when a fish is brought to the surface more rapidly than it can remove gas from its swim bladder or can equilibrate gas levels in its circulatory system. Rapid decompression can occur in recreational or commercial fishing; more recently, the phenomenon is being observed in fish passing through turbines (Brown et al. 2009). Few studies have quantified barotrauma due to rapid decompression in juvenile salmonids. D'Aoust and Smith (1974) used four different pressure change scenarios (from 59.2, 41.3, or 28.1 to 14.7 psia and from 14.7 to 2.3 psia, creating exposure ratios from 1.91 to 6.0) to quantify barotrauma in fingerling steelhead (*Salmo gairdnerii*) and coho salmon (*Oncorhynchus kisutch*). Recently, Brown et al. (2009) simulated turbine passage of seaward-migrating juvenile Chinook salmon acclimated to atmospheric pressures of 14.7, 19.0, 23.4, or 32.1 psia. Acclimated fish were subsequently exposed to rapid decompression (simulating turbine passage; STP) with nadirs ranging from 1.2 to 2.8 psia (a ratio of 27.0 to 5.3) Rapid decompression and resulting barotrauma were examined in physoclistous Centrarchids (*Micropterus salmoides*) by Feathers and Knable (1983). These researchers used limited acclimation pressures (four scenarios, in which the largest exposure ratio was 3.7; a decrease in pressure from 53.7 to 14.7 psia.). All of these researchers observed that barotrauma-related injuries increased as the ratio of acclimation to exposure pressure increased. Although this information is insightful, information about fish barotrauma along a wide and continuous range of pressure changes is needed to predict mortality of fish passing through existing turbines and to optimally design and operate turbines. We hypothesized that barotrauma would increase in juvenile Chinook salmon as the ratio of acclimation to exposure pressure increased (over a continuous range of nadirs).

Although it is known that barotrauma is linked to the ratio of acclimation to exposure pressures, it is unclear how the rate of pressure change influences barotrauma. Similar to a scuba diver who must ascend slowly to remove excess gas dissolved in blood and tissues (to prevent the bends), fish must decompress at a rate that allows gas to be expelled via the blood at the gills (in physoclists) and by 'spitting' (observed in salmonids and other physostomes) gas from the swim bladder to prevent barotrauma. Rates of pressure change (i.e., decompression) recorded within Columbia River turbines are highly variable, with recorded ranges from 9 psi/s at Bonneville Dam to as fast as 839 psia/s at Ice Harbor Dam (Carlson et al. 2008). However, it is not known how the occurrence of barotrauma will change with rates of pressure change. We hypothesized that barotrauma would be higher when fish were exposed to greater rates of pressure change during simulated turbine passage.

Barotrauma occurs when free gas in the swim bladder and other tissues and organs expand during rapid decompression and when a decrease in pressure decreases the solubility of gas in blood increasing the amount of free gas (Feathers and Knable 1983). However, it is unclear how the concentration of gas within the surrounding aquatic environment affects the incidence of barotrauma. Barotrauma caused by decompression is characterized by expansion in the volume of any gas-bearing organ, such as the swim bladder, or free gas within the body of a fish or the release of gas from solution into the blood. Gas freed from solution within the vasculature can disrupt organ function. Increase in vessel internal pressure can cause vessel rupture and subsequent hemorrhage. Common causes of immediate death are suffocation (from emboli in the gills), hemorrhage, and disruption of heart function (Brown et al. 2009). Because the tension of gas both free and in solution in a fish's body is in equilibrium with that of the surrounding water, fish may be at greater risk of barotrauma when their habitats are saturated or supersaturated with total dissolved gas, as is common in rivers with hydroelectric dams. This is particularly true in cases where spilled water is used as an element of strategies to pass downstream migrants around the dams (Ryan et al. 2000). Total dissolved gas saturation levels can be highly variable in the Columbia and

Snake rivers, typically ranging from 100% to 120% (Columbia River DART 2010) but have been observed as high as 143% (Ebel 1969; Beiningen and Ebel 1970, 1971; Meekin and Allen 1974; Backman and Evans 2002). In the study described in this chapter, we hypothesized that barotrauma would increase as levels of total dissolved gas increase.

Experimental Methods

Factors Relating Mortal Injury of Untagged Fish to Rapid Decompression

Fish Acquisition

Hatchery-reared juvenile Chinook salmon, *O. tshawytscha* (*n* = 5713, median length = 122 mm, range 71 to 205 mm; median weight = 19.3 g, range 3.7 to 134 g), were exposed to STP treatments between March 7, 2007, and March 6, 2010. The fish were either acquired as fry or hatched and reared from eggs at the PNNL Aquatic Research Laboratory (ARL).

Testing of juvenile salmon was conducted in the hyper/hypobaric chambers described in Chapter 1. During testing, ambient well water (median temperature = 17.0°C; range 15.8 to 17.9°C) was pumped to the chambers after the total dissolved gas (TDG) levels were elevated. A pack injection column (Point Four Systems Inc., Richmond, British Columbia) was used to elevate TDG to two levels: 115% (median = 114.8, range 112.7 to 118.2, *n* = 3533) and 125% (median = 124.5, range 122.2 to 127.5, *n* = 2180). Total dissolved gas (TDG) was monitored with sensors installed within each chamber (Model T507, In-Situ Inc., Fort Collins, Colorado; ±1.5 mm Hg accuracy). Levels of TDG were recorded on a data logger (Campbell Scientific, Logan, Utah) controlled by a program written in CRBasic and implemented via LoggerNet. Treated water was supplied to all chambers at a continuous rate of 7.6 L/min with a flow control accuracy of ±0.95 L/min, similar to the conditions described in Chapter 1.

Acclimation Prior to Pressure Exposure and Simulated Turbine Passage

Juvenile Chinook salmon were marked and loaded into chambers as described in Chapter 1. Acclimated pressures were equivalent to the absolute pressures that would exist at three different depths in fresh water, given a standard atmospheric pressure of about 14.7 psia: 5 ft (16.9 psia; *n* = 1193), 15 ft (21.2 psia; *n* = 3786), and 25 ft (25.5 psia; *n* = 734). Fish were held at acclimation pressure for 16–24 h prior to testing to allow ample time to attain neutral buoyancy and equilibration of gas tensions in bodily fluids and tissues. The determination of buoyancy, exposure to STP, and necropsy procedures were conducted using observations and video equipment described in Chapter 1. Although we tested more than 5713 fish, a small proportion of these fish (6.9% were negatively buoyant, and < 0.1% were positively buoyant) never gained neutral buoyancy following 16 h of acclimation. Given the results described in Chapter 1 and the assumption that in-river fish are neutrally buoyant when approaching hydroelectric facilities (due to energy conservation in wild systems), we included only neutrally buoyant fish for the analyses documented in this chapter.

Exposure Pressures and Rate of Pressure Change

Exposure pressures (i.e., the nadir) during STP ranged from 0.93 to 21.0 psia, with a median of 6.4 psia. The rate of pressure change (i.e., rate of decompression) during STP ranged from 110 to 562 psia/s (median = 203 psia/s).

Histology

To further investigate the extent of injury from STP exposure, 40 fish that died during or shortly after STP were randomly selected for examination using histological techniques following Elston et al. (1997) and Brown et al. (2009). Briefly, each fish was fixed in neutral buffered formalin with the visceral cavity opened by incision. Specimens were subsequently submitted to the AquaTechnics laboratory (Sequim, Washington) for histological processing and examination. To process the fish for histological examination, three transverse sections were made through each fish, from locations anterior to posterior through the peritoneal cavity. One frontal section was also made through the mid- to lower head region. A single section was cut from each histological block, processed by conventional paraffin embedding, and stained with hematoxylin and eosin (Brown et al. 2009). After organs and tissues were processed, each slide was examined for abnormal structures and lesions, especially as they may relate to structural changes in organs and tissues resulting from barotrauma.

Mortal Injury

After they were exposed to STP, many fish died within a few minutes or received pressure-related injuries (barotrauma) sufficient to cause eventual mortality. It is not always feasible to hold fish following rapid decompression testing, and the conditions in which fish could be held could be highly variable (pressure, temperature, total dissolved gas, or other conditions may vary). Subsequently, a metric that predicted mortal injury, derived by McKinstry et al. (2007), was used as the response variable in this study instead of mortality and a multitude of different injury types. The mortal injury metric was derived by analysis of a large data set of fish exposed to rapid decompression. The metric associated fish that died within minutes of rapid decompression with the injuries that were observed during necropsy. The injuries seen most often in fish that died (determined using odds ratios, Fisher's exact tests, and stepwise logistic regression modeling using AIC [McKinstry et al. 2007]) were included in the metric. These injuries included exothalmia (eye-pop); 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. Fish that died were also considered to be mortally injured. Although emboli in the pelvic fins appears to be an injury that would not be associated with mortality, among the fish that had this malady ($n = 284$), 187 or 65.8% died during or within a few minutes of exposure to rapid decompression. Thus, emboli in the pelvic fins acts as an externally observable predictor of mortality. Therefore, mortal injury served as the endpoint and response variable for these analyses, and fish with any one of these eight injuries present, or fish that were dead shortly following testing (within ~10 min), were classified as mortally injured. Although other injuries were noted which could lead to delayed mortality or increased chance of predation, they were not highly associated with mortality shortly after STP, and therefore were not included in this analysis.

Statistical Design and Analysis

Together, 5713 hatchery-reared juvenile Chinook salmon were exposed to STP. Mortal injury for each test fish served as the dependent or response variable. Six explanatory variables served as the independent variables:

- rate of pressure change (ROC)

- total dissolved gas (TDG) level

- ratio of acclimation to exposure (nadir) pressures with natural log applied (LRP)

- fish length

- fish weight

- condition factor.

Pressure Ratio (LRP)

Rapid decompression testing simulates the effects of the rapid drop in pressure from acclimation depth to nadir pressure that occurs during turbine passage. Our study was designed originally as a factorial experiment, which included depth of acclimation as an experimental factor variable; specific levels of 5, 15, and 25 ft corresponded to depth-specific pressures of 16.9, 21.2, and 25.5 psia, respectively. The nadir pressure (lowest exposure pressure) was recorded in psia during the test on a continuous scale and was to be used as another covariate in the statistical model.

Preliminary analysis suggested that the probability of mortal injury was affected by the nadir pressure but modified by the pressure to which fish were acclimated prior to testing. This finding is consistent with Boyle's law that describes the physical relationship between pressure, volume, and temperature in a closed system. When temperature is held constant, Boyle's law may be expressed as

$$p_A V_A = p_N V_N \qquad (2.1)$$

where
p_A = acclimation pressure
p_N = nadir pressure
V_A = volume of air contained in the swim bladder of a fish at acclimation depth
V_N = volume of air contained in the swim bladder of a fish at nadir pressure.

Rearranging Equation (2.1),

$$\frac{p_A}{p_N} = \frac{V_N}{V_A} \qquad (2.2)$$

Equation (2.2) indicates the ratio in pressure change from acclimation depth to nadir pressure is inversely proportional to the expected ratio of change in the air volume in the swim bladder of test fish. Application of Equation (2.2) allows the two model terms for pressure at depth of acclimation and the nadir pressure to be combined into a single term in the statistical model as shown in Equation (2.3):

$$\text{LRP} = \ln\left(\frac{p_A}{p_N}\right) \qquad (2.3)$$

Using the logarithm of the pressure ratio converts the multiplicative effects of p_A and p_N to a linear effect on the log scale. Therefore, LRP is the log ratio pressure change of acclimation pressure to nadir pressure.

Condition Factor

Condition factor (measure of energy storage) was calculated using

$$C = (W/L^3) \times 100{,}000 \tag{2.4}$$

where W = the weight of the fish in grams, L = the fork length of the fish in millimeters, and 100,000 is a scaling constant to convert small decimals into mixed numbers for ease of comprehension (Winter 1983).

Statistical Models

Mortal injury was modeled 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

$$y_{ij} = \begin{cases} 1 \text{ if fish died/injured} \\ 0 \text{ otherwise} \end{cases} \tag{2.5}$$

Concepts of *r* or *r²* are not very meaningful in binary (0,1) regression, and an alternative expression of model performance was necessary. Logistic model performance was evaluated using a measure called *area under the curve* (AUC; Hosmer and Lemeshow 1999). 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 or stochastic 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 2.1), which plots true positive rate versus false positive rate. We compared AUC values between alternative logistic models to identify their relative levels of performance. The AUC value is the area under the curve inside the unit square.
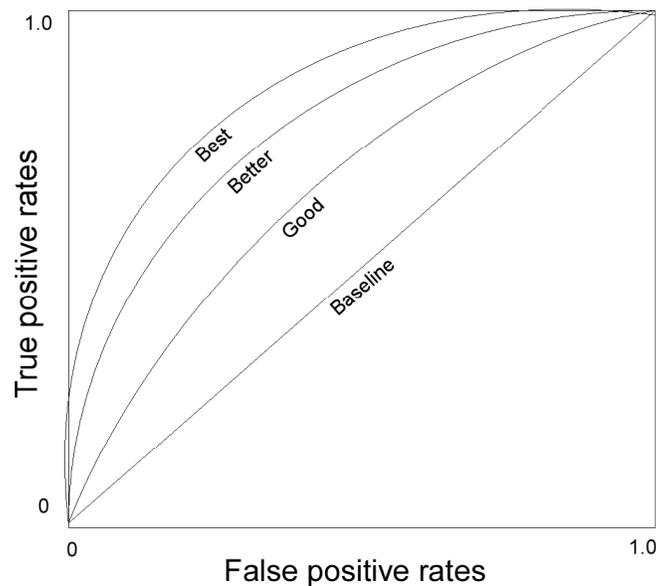
Figure 2.1. Area under the curve (AUC) with alternative model performance curves.

A Comparison of Hatchery-Reared and Seaward-Migrating Fish

Fish Acquisition

The purpose of this investigation was to determine whether the probability of mortal injury for seaward-migrating juvenile Chinook salmon can be described by the same model as that for hatchery-reared juvenile Chinook salmon. Seaward-migrating juvenile Chinook salmon ($n = 1119$), median length of 105.0 mm (range = 76 to 184 mm), median weight of 12.5 g (range = 4.4 to 61.2 g) were collected daily from the Columbia River using the juvenile bypass facility at McNary Dam and held overnight, unfed, in a 125-L circular container with flow-through ambient river water. Throughout the testing period, the water temperature was a median of 20.4°C (range = 13.3 to 21.8°C).

Hatchery-reared juvenile Chinook salmon ($n = 2217$), median length of 115.0 mm (range 71 to 135 mm), median weight of 16.1 g (range 3.7 to 29.8 g) were either acquired as fry or hatched and reared from eggs at the PNNL ARL. While being reared at the ARL, test fish were held in 1100-L circular holding tanks with flow-through ambient well water (~17°C) and nourished with Biodiet moist pellets (Bio-Oregon, Longview, Washington) ad libitum. Water temperature during the acclimation and testing was a median of 17.0°C (range 16.5 to 17.6°C).

Exposure Pressures and Rate of Pressure Change

For seaward-migrating fish, exposure pressures during STP ranged from 1.1 to 18.6 psia, median 6.7 psia; the rate of pressure change during STP ranged from 107 to 263 psia/s (median 176 psia/s). Test fish were exposed to one of two TDG levels: 115% (median = 115.5%, $n = 1112$) and 125% (median = 125.7%, $n = 7$). Exposure pressures (nadir) for hatchery-reared fish ranged from 0.9 to 21.3 psia (median 6.2 psia), and the rate of pressure change during STP ranged from 121 to 562 psia/s (median 200 psia/s). During acclimation and testing, TDG was held constant at one of two levels for each test fish: 115% (median = 114.7, $n = 750$) and 125% (median = 124.6%, $n = 1467$). Table 2.1 summarizes the total length, mass, temperature, TDG, nadir, and rate of change for data for hatchery and seaward migrating fish.

Although fewer trials of seaward-migrating fish ($n = 169$)[1] were performed than those of hatchery-reared juvenile Chinook salmon ($n = 321$), the ranges of LRP conditions were similar (Figure 2.2). Therefore, all trials were used in the subsequent test of congruence between hatchery and seaward-migrating response models.

Statistics Design and Analysis

The proportion of fish mortally injured within each trial served as the dependent or response variable. The natural log of the ratio of acclimation to nadir pressures (LRP) was the explanatory variable. Analysis of deviance was used to test whether the model for hatchery-reared fish adequately described the response model for seaward-migrating fish. Specifically, these statistics were carried out in a manner similar to those of the previous section in which mortal injury serves as the response variable and LRP the predictor variable. However, this comparison was carried out between the two fish treatments—seaward-migrating and hatchery-reared. In addition, we used an analysis of deviance model to compare linear regressions of acclimation depth versus LRP while comparing the two treatments. Other variables such as

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[1] A trial equates to the fish within each test chamber.

condition factor, fish size, and ROC were omitted from these analyses because they were shown in the previous analysis to have limited predictive power. Further, the intention of this analysis was to compare the differences between hatchery-reared and seaward-migrating fish exclusively without the confounding effects of other independent covariates.

Table 2.1. The length and weight characteristics of juvenile Chinook salmon exposed to simulated turbine passage. Temperature, total dissolved gas, pressure nadir, and rate of pressure change to which the two groups were exposed are detailed.

Characteristic	Hatchery (*n* = 2217)			Seaward migrating (*n* = 1119)		
	Median	Min	Max	Median	Min	Max
Length (mm)	115.0	71.0	135.0	105.0	76.0	184.0
Weight (g)	16.1	3.7	29.8	12.5	4.4	61.2
Temperature (°C)	17.0	16.5	17.6	20.4	13.3	21.8
Total dissolved gas (%)	124.2	112.7	127.5	115.5	111.3	125.7
Nadir (psia)	6.2	0.9	21.3	6.7	1.1	18.6
Rate of pressure change (psia/s)	190.0	121.0	562.0	176.0	107.0	263.0
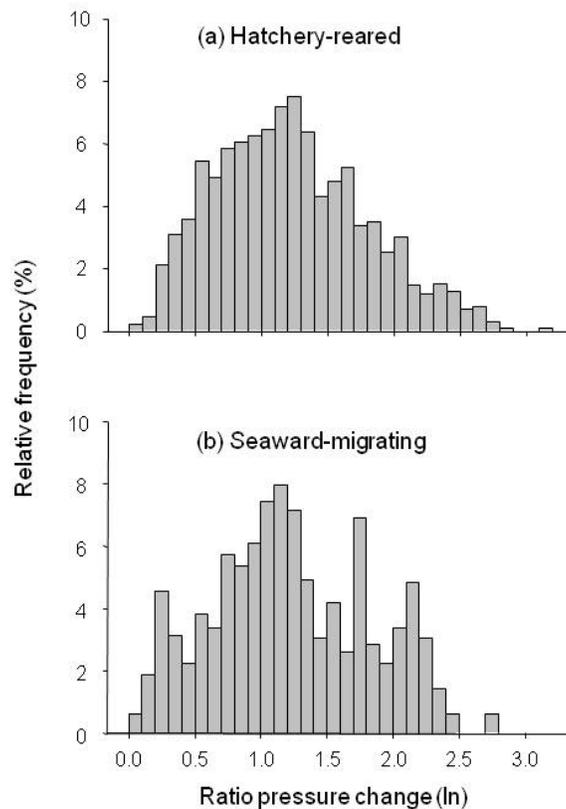
Figure 2.2. Ratio of pressure change (with natural log applied) exposure conditions for hatchery-reared and seaward-migrating Chinook salmon.

Results

Factors Associated with Mortal Injury

All the main treatment factors of LRP ($P < 0.001$), TDG ($P < 0.001$), condition factor ($P < 0.001$), ROC ($P = 0.0003$), and the interaction effects of TDG by condition factor ($P < 0.001$), and LRP by TDG ($P = 0.0005$) were significant predictors of mortal injury in juvenile Chinook salmon (Table 2.2). Neither length ($P = 1.0$) nor weight ($P = 1.0$) was a significant variable. The main model plus the two interactions explained 40.20% of the deviance in the data. LRP explained by far the most deviance in the model (39.10%), while all of the other variables combined explained less than 1% of the deviance in the model (TDG = 0.31%, condition factor = 0.31%, ROC = 0.14%, TDG by condition factor interaction = 0.18%, and LRP by TDG interaction = 0.13%). The interactions among condition factor, LRP, and TDG can be visualized in Figure 2.3; the lines in the upper panel (where TDG = 115) slope to the right, indicating that mortal injury is higher for fish with a lower condition factor. In the lower panel (where TDG = 125), the lines slope slightly to the left, indicating the mortal injury is higher for fish with higher condition factor. Because TDG explained little of the variance in the model, this inconsistent relationship between condition factor and other explanatory variables for the two levels of TDG is likely an indication of the interaction among TDG, LRP, and condition factor.

Similar to the analysis of deviance results, the area under the curve analysis also indicated that LRP is the most influential factor in determining the likelihood of mortal injury. The model containing all significant effects and interactions had an AUC score of 0.9003 (Figure 2.4). However, the simplest model that contains only LRP had an AUC of 0.8976, thus explaining almost all of the deviance in the model. This single-variable model with LRP appears to be an adequate predictor of mortal injury.

An analysis of deviance of this simplified model indicates that LRP is a significant ($P < 0.0001$) predictor of mortal injury (Table 2.3 and Figure 2.5), explaining 39.10% of the deviance in the model. The equation for predicting mortal injury given LRP is

$$\text{Probability of mortal injury} = \frac{e^{-5.56+3.85*LRP}}{1+e^{-5.56+3.85*LRP}} \tag{2.6}$$

Table 2.2. Sequential analysis of deviance table of the factors associated with the mortal injury of juvenile Chinook salmon with respect to simulated turbine passage. The final model explained 39.10% of the deviance in the data; see text for details. Factors include LRP = natural log of the ratio of acclimation to exposure pressure, TDG = total dissolved gas (%), CF = condition factor, and ROC = the rate of pressure change (psi/s) during simulated turbine runner passage. Factors to the right of the "|" symbol were already in the model when the factor to the left of the symbol was examined.

Source	df	Deviance	Mean deviance	F	P
Total	5712	7518.7			
Main effects					
LRP	1	2939.5	2939.5	3666	< 0.0001
TDG | LRP	1	23.5	23.5	29.5	< 0.0001
CF | LRP + TDG	1	23.1	23.1	27.4	< 0.0001
ROC | LRP + TDG + CF	1	10.2	10.2	12.9	0.0003
Interactions					
TDG × CF | LRP + TDG + CF + ROC	1	13.4	13.4	17	< 0.0001
LRP × TDG | LRP + TDG + CF + ROC + TDG × CF	1	9.6	9.6	12.2	0.0005
Error	5706	4499.3	0.79		

2.10
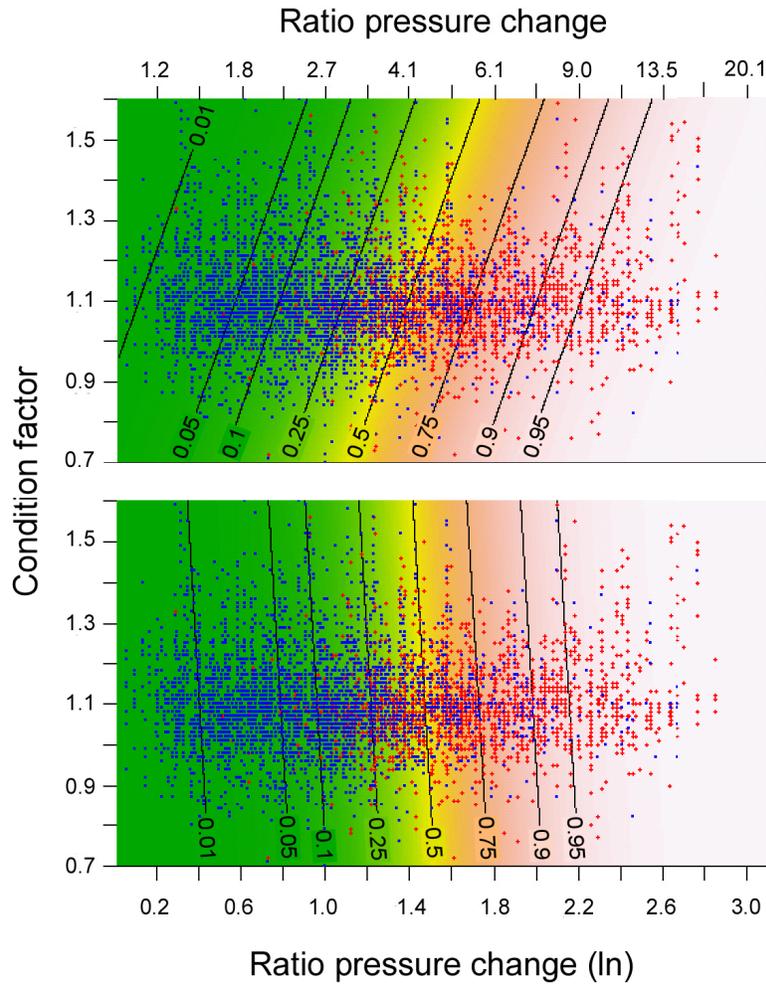
Figure 2.3. Estimated probability of mortal injury versus the natural log of the ratio of acclimation to exposure pressure (LRP) and condition factor (CF) for juvenile Chinook salmon when exposed to total dissolved gas (TDG) levels of 115% (upper panel) or 125% (lower panel). Green indicates the lowest mortal injury and white indicates the highest for the various combinations of LRP, TDG, and CF. Data points for fish tested are shown in either red (for fish that were mortally injured) or blue (for fish that were not mortally injured). Note that in the upper panel, lines lean to the right indicating that mortal injury is higher for fish with lower CF, while on the lower panel, lines lean slightly to the left, indicating the mortal injury is higher for fish with higher CF.
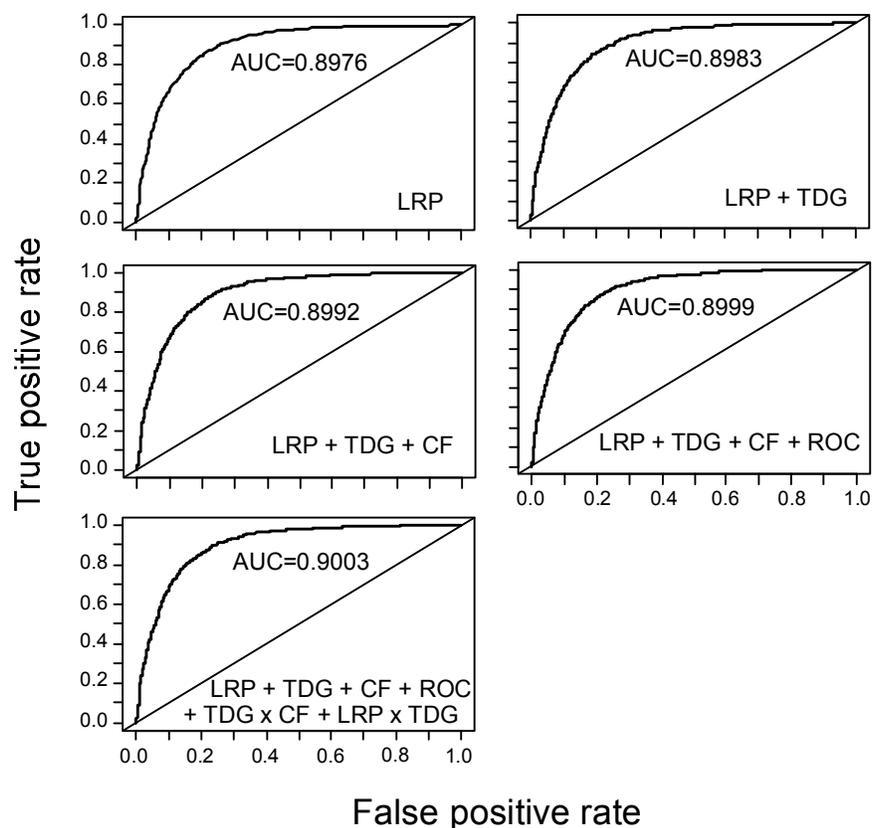
Figure 2.4. Area-under-the-curve (AUC) plots of the predictive value from each progressive model and the actual mortal injury outcome. Factors include LRP = natural log of the ratio of acclimation to exposure pressures, TDG = total dissolved gas (%), CF = condition factor, and ROC = the rate that pressure changed (psi/s) during simulated turbine passage.

Table 2.3. Analysis of deviance of the factors associated with the mortal injury of juvenile Chinook salmon with respect to simulated turbine passage. The simplified model explained 39.15% of the deviance in the data. LRP = natural log of the ratio of acclimation to exposure pressures.

Source	df	Deviance	Mean deviance	*F*	*P*
Total	5712	7518.7			
Main effects					
LRP	1	2939.5	2939.5	3666	< 0.0001
Error	5711	4579.2	0.8		
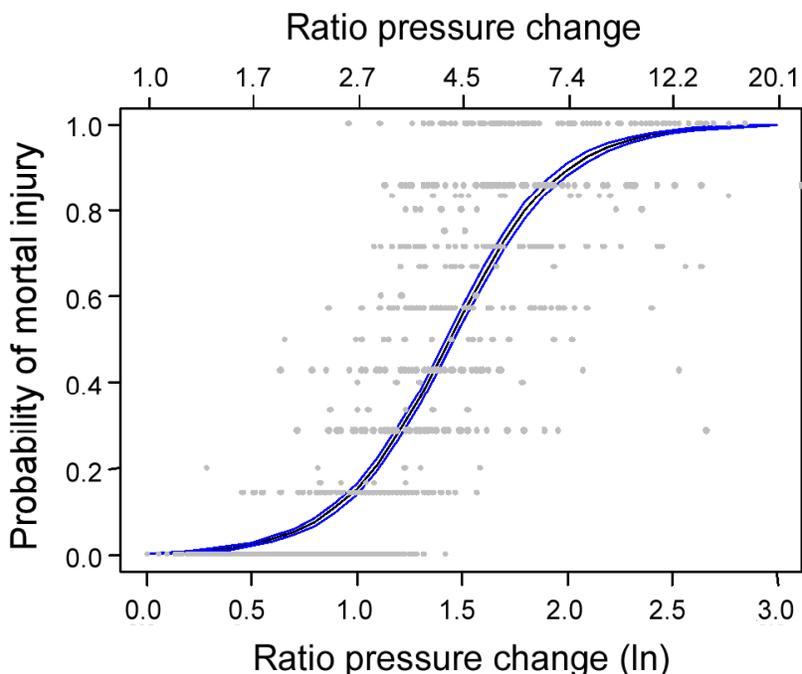
Figure 2.5. Probability of mortal injury along a range of LRP (natural log of the ratio of acclimation to nadir pressures) for juvenile Chinook salmon. The 95% confidence interval is shown on either side of the regression line (center line). Each dot on the graph indicates an individual fish exposed to simulated turbine passage.

Histology

Injuries observed during the histological examination of juvenile Chinook salmon included hemorrhaging of the heart and vasculature, eversion within the gastro-intestinal system, and dilation of gills (Table 2.4). Hemorrhaging, the most common injury, was observed in an average of 42.0% of fish that died after STP. Hemorrhaging was most commonly noted in vasculature associated with the swim bladder (in 55.0% of fish) followed by hemorrhaging in the kidney or caudal vein (45.0%), other areas in the peritoneum (35.0%), in the pericardial cavity (30.0%), and in the brain (5.0%). Hemorrhaging within the swim bladder appeared to result from rupture of the capillaries along its inner walls. This type of hemorrhaging was seen only in fish exposed to LRP greater than 1.5.

The primary site of hemorrhage in the kidney was rupture of the caudal vein. Rupture of this vein was also the likely cause of much of the hemorrhaging seen within the peritoneum that could not be isolated. This type of hemorrhage was most common among fish exposed to LRP greater than 1.5.

Hemorrhaging within the pericardial sac was observed between the auricles, ventricle, or conus arteriosus and in the pericardial membrane. The most common site was between the conus arteriosus and the pericardial membrane. This type of hemorrhaging was commonly noted across the range of LRP.

Table 2.4. Occurrence of three different types of internal injury in juvenile Chinook salmon as determined by histological examination. The number of fish with each type of injury is indicated at different levels of LRP (ratio pressure change with a natural log applied). A sum of the fish with each type of injury and the percentage of fish with each type of injury also are shown.

| LRP range | n | Hemorrhaging | | | | | | Gastro-intestinal eversion | | | Dilation |
		Brain	Pericardial cavity	Swim bladder	Peritoneal cavity	Head kidney	Trunk kidney	Pharynx	Stomach	Pyloric caecae	Gills
0.50–1.00	2	0	1	0	1	0	0	0	0	0	0
1.00–1.50	3	0	2	0	0	0	0	0	0	0	0
1.50–2.00	13	1	4	13	6	13	13	2	2	13	2
2.00–2.50	21	1	4	9	6	5	5	2	2	2	4
2.50–3.00	1	0	1	0	1	0	0	0	0	0	0
Sum	40	2	12	22	14	18	18	4	4	15	6
Fish with injury (%)		5.0	30.0	55.0	35.0	45.0	45.0	10.0	10.0	37.5	15.0

Hemorrhaging of the brain was one of the least common types of hemorrhaging noted during histological examination (seen in 5.0% of fish). In a single fish, a ruptured arteriole was found at the surface of the meninges (membrane covering the brain). A ruptured capillary was observed in the meninges of an additional fish, resulting in localized hemorrhage in the area adjacent to the arteriole rupture. Both of these fish were exposed to LRP greater than 1.5 (Table 2.4).

Eversion of sections of the digestive tract were the second most common category of injury noted during histological examination, being observed in as much as 37.5% of fish. When observed, a relatively lower portion of the gastro-intestinal tract was pushed in an anterior direction into the upper digestive tract and a trailing section of lower digestive tract was pulled into the everted section. This type of injury was most common among fish exposed to higher (>1.5) LRPs. Eversion of the pyloric caecae was the most common type of eversion noted. Eversion of the stomach and pharynx were each seen in 10.0% of fish.

Dilation of the gills is an indication that emboli were present. Multiple primary lamellae or filaments of gill were observed with dilated veins, resulting in a cross-sectional cavity. Dilation of the gills was more common (15.4–19%) among fish exposed to higher (>1.5) LRPs (Table 2.4).

A Comparison of Hatchery-Reared and Seaward-Migrating Fish

Overall, the relationship between mortal injury and LRP were not statistically different ($P = 0.0541$) between hatchery-reared and seaward-migrating subyearling Chinook salmon (Table 2.5). In addition, the intercept coefficient of the regression model for seaward-migrating fish was not significantly ($P = 0.1961$) different from the model for the hatchery-reared fish. However, the LRP coefficient was significantly ($P = 0.0367$) different between the two models (Table 2.6). The differences between the two models can be seen where the red and blue lines deviate at LRP of approximately 1.5–2.5. In this range, the mortality for hatchery-reared fish appears to be slightly higher than that of seaward-migrating fish at the same ratio of pressure change (Figure 2.6).

The slight difference between the hatchery-reared and the seaward-migrating models can also be visualized when data are broken down for each of the three acclimation depths (Figure 2.7). The deviation between the lines for the two models is present at the lower exposure nadirs for fish exposed to all depths. However, the difference appears to be slightly higher for fish acclimated to 15 ft and 25 ft than for fish acclimated to 5 ft.

Table 2.5. Analysis of deviance for the test of congruence between seaward-migrating and hatchery-reared subyearling Chinook salmon. LRP = natural log of the ratio of pressure change during simulated turbine passage.

Source	df	Deviance	Mean Deviance	F	P (>F)
Total$_{COR}$	489	2311.2222			
LRP	1	1462.2135			
Source effects	2	10.1286	5.0643	2.934	0.0541
Error	486	838.8801	1.7261		

Table 2.6. Coefficients of the model including hatchery-reared and seaward-migrating subyearling Chinook salmon. I(S) is an indicator function for the fish run type. LRP = natural log of the ratio of acclimation to nadir pressures.

Parameter	Value (SE)	$P\ (>|Z|)$
Intercept	5.4236 (0.2190)	<0.0001
LRP	3.4974 (0.1504)	<0.00001
Intercept: I(S)	0.4779 (0.3697)	0.1961
LRP: I(S)	-0.5147 (0.2464)	0.0367
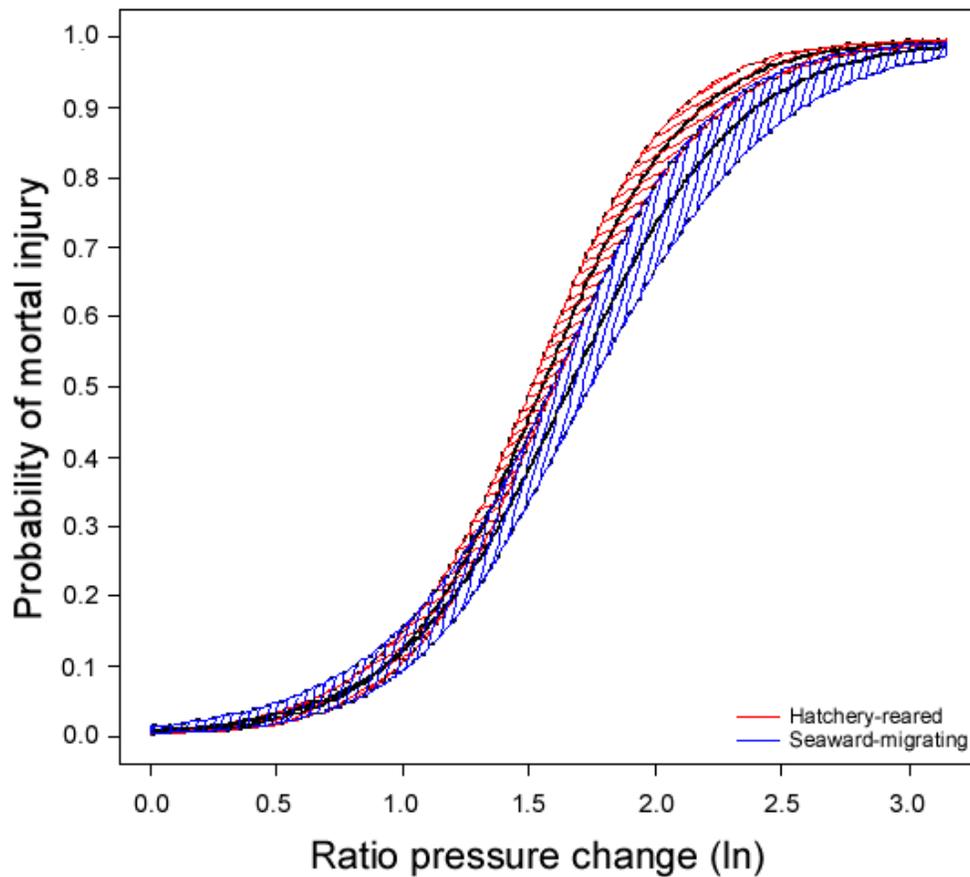
Figure 2.6. Overlays of fitted logistic probability curves of mortal injury along a range of LRP (natural log of the ratio of acclimation to nadir pressures) for juvenile Chinook salmon that were either hatchery-reared (red) or seaward-migrating (blue). The shaded area is the 95% confidence interval for the regression line.
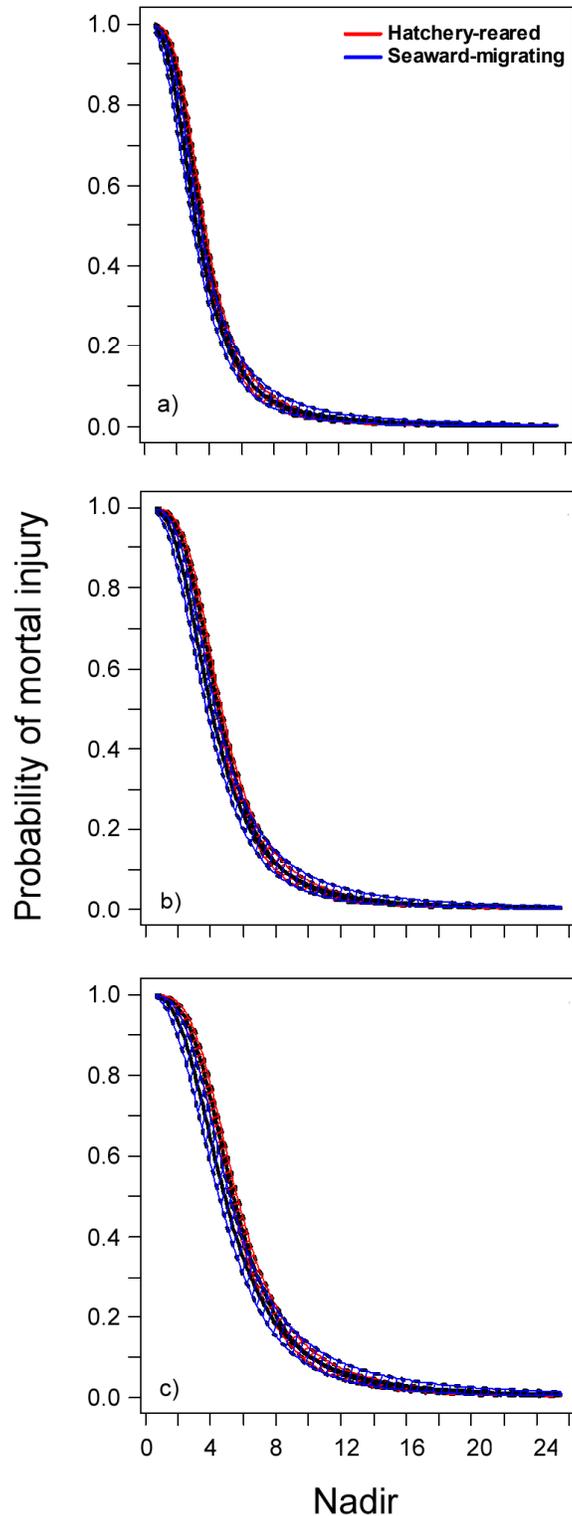
**Figure 2.7**. Overlays of fitted logistic probability curves of mortal injury along a range of nadir for juvenile Chinook salmon that were either hatchery-reared (red) or seaward-migrating (blue). Data are shown for fish acclimated to the static pressure equivalents of 5 ft (a), 10 ft (b), or 15 ft (c) of depth. The shaded area is the 95% confidence interval for the regression line.

Discussion

Factors Associated with Mortal Injury

The main factor associated with mortal injury of juvenile Chinook salmon during simulated turbine passage was the natural log of the ratio between acclimation and nadir pressures. Specifically, the likelihood of mortal injury increased with higher pressure ratios. As we hypothesized, the probability of barotrauma-related mortal injury increased in juvenile salmon as the ratio of acclimation pressure to exposure pressure increased. However, instead of increasing linearly, barotrauma increased in a sigmoidal fashion, where the largest increases in mortal injury were observed in the middle range of pressure ratios tested. The sigmoid nature of the response curve reflects the functional form of the likelihood equation fit to the experimental data. It was observed that at the lower range of pressure ratios tested, the probability of mortal injury was low, while at the upper range of pressure ratios, the probability of mortal injury was almost 1.0, as indicated by the narrower confidence intervals in these areas.

Researchers have examined the influence of pressure change in barotrauma-related injury; however, previous research has been confined to examining a limited range of pressure change exposures. For example, Brown et al. (2009) examined the relationship between mortality and several injury types in juvenile Chinook salmon exposed to simulated turbine passage. They exposed fish to four distinct levels of pressure change (from acclimation pressures of 14.7, 19.0, 23.4, or 32.1 psia to exposure pressures of 1.2–2.8 psia; a range of LRP from 1.7 to 3.3. Rummer and Bennett (2005) examined decompression of red snapper (*Lutjanus campechanus*) that simulated recreational angling. They examined mortality and injury that resulted from a slow decrease (1.5 psia/s) in pressure from acclimations of 58.7, 88.2, or 176.4 psia to surface pressure (14.7 psia); a range of LRP from 1.4 to 2.5. In contrast, the research presented here is the first to expose fish to a wide range of pressure changes (from no pressure change to an LRP of 3.12; ratios of pressure change from 0.0 to 22.6) and to describe the relationship between pressure change using the derived variable LRP and barotrauma-related injury using the derived variable mortal injury.

Fish used in the current research were acclimated to the pressures present at 5, 15, and 25 ft of depth. The depth at which these fish approach a turbine is important considering acclimation depth, or depth at which fish are neutrally buoyant prior to turbine entry, determines the numerator of the ratio of acclimation and exposure pressures. There are some data for the depth at which juvenile salmonids (*Oncorhynchus* sp.) occur during migration through the Columbia River hydropower system. For example, Beeman and Maule (2006) indicated that mean depths of juvenile steelhead (*O. mykiss*) ranged from 6.6 ft in the Snake River to 7.5 ft close to the McNary Dam forebay in the Columbia River. In addition, the mean depths of yearling Chinook salmon ranged from 5.0 ft in the Snake River to 10.5 ft near the forebay of McNary Dam. Faber et al. (2005) observed suspected juvenile yearling and subyearling Chinook salmon and steelhead in the upper 20 ft of the water column in the forebay of The Dalles Dam, but it was not uncommon to observe them at depths of 40 ft in the forebays of Columbia River dams (Faber et al. 2001). Similar depths of juvenile salmonids were observed by Dauble et al. (1989) in the Hanford Reach of the Columbia River. However, in all these cases it is not clear if these fish were neutrally buoyant at these depths.

The importance of knowing a fish's buoyancy cannot be understated (see Chapter 1) because the depth at which fish are neutrally buoyant determines how much air, in terms of mass, is found within a

fish. This is the case because fish at greater depth require a larger mass of air in their swim bladder to compensate for air volume compression with depth (see Boyles' law presented earlier) to achieve the swim bladder volume necessary for neutral buoyancy. More important, the greater the acclimation depth of a fish, the higher the probability of mortal injury for any specific nadir pressure. Brown et al. (2009) showed that the ratio of acclimation to nadir pressures is the best explanatory variable for prediction of the probability of mortal injury in juvenile Chinook salmon, not acclimation depth equivalent pressure or nadir pressure alone or the absolute change in pressure between the two pressures. For example, two fish acclimated to a depth of 15 ft (21.2 psi) will have very different probabilities of mortal injury following turbine passage when one fish is exposed to a nadir of 2 psi and the other 10 psi. The ratio of pressure change is 10.6 (21.2/2) for the first scenario and 2.12 (21.2/10) for the latter. In the current study, we determined that a 10.6 ratio of pressure change leads to severe damage and a high probability of mortal injury, while a ratio pressure change of 2.12 leads to infrequent physical injury and a low probability of mortal injury. Furthermore, over the range of the ratios of acclimation to nadir pressures we tested, we observed that the greatest amount of variability in the occurrence of mortal injury occurs in the middle range of LRP.

The current research also highlights the importance of determining the depth of neutral buoyancy prior to turbine passage, as well as determining the nadir pressures to which fish are exposed during turbine passage. Although little information exists for the former, a device called the Sensor Fish has been used to determine the pressures present within existing Columbia River basin turbines to which fish are exposed during turbine passage (Deng et al. 2007; Carlson et al. 2008). The Sensor Fish records pressures, three components of linear acceleration (up–down, forward–back, and side-to-side), and three components of angular velocity (pitch, roll, and yaw) as it passes through turbines. In some Sensor Fish examples, nadirs (lowest exposure pressure) below vapor pressure have been measured (Columbia River turbine examples; Carlson et al. 2008), but most range between 5 and 29 psia. These data are important because nadir is the other factor that determines LRP (the first being the acclimation depth equivalent pressure). Sensor fish data can be used to estimate the nadir pressures and other features of the changes in pressure fish experience during turbine passage. This information, coupled with our response data, permits evaluation of the risk of mortal injury from barotrauma for the population of juvenile Chinook salmon passing through turbines. Other researchers have recognized the need for better data to describe the pressure time history exposure for fish passing through turbines, the physiological state of fish at turbine entry, and behavior during turbine passage (reviewed in Coutant and Whitney 2000).

Variables other than LRP were found to be statistically significant factors accounting for variability in observations of the probability of mortal injury. However, these variables were found to only slightly increase the predictive power of equations relating probability of mortal injury to conditions of exposure or characteristics of test fish during simulated turbine passage. For example, TDG was found to be a statistically significant variable to help explain the variability observed in probability of mortal injury but explained very little of the deviance in the model when compared to LRP. The effects of elevated TDG on fish health have been studied extensively in the Columbia and Snake rivers, and high levels of TDG have been blamed for fish kills in these aquatic habitats (Ebel 1969; Ebel et al. 1975). Total dissolved gas in the Columbia River varies spatially and temporally and tends to increase with the volume of water spilled. In 1999, 6 km downstream of Ice Harbor Dam, Beeman and Maule (2006) found TDG values that varied between 105.2% and 131%. Further, historically high levels (exceeding 125%) of gas have been observed to occur within large areas of the Columbia River basin, usually around May to mid-August (Ebel 1969), as a result of increased spill designed to facilitate juvenile downstream migration

(Johnson et al. 2007). This range is important because many researchers have observed increases in mortality once fish are exposed to TDGs above 125% (Ebel et al. 1971). Total dissolved gas has been a concern for dam operators as they aim to improve water quality for resident fishes (Williams 2008). Increased TDG likely leads to more gas within the body of fish and therefore may increase the potential for increased mortal injury for turbine-passed fish. Based upon observations of elevated TDG levels (as detailed above), our exposure of fish to levels in the 115% to 125% range seems reasonable. We found that the contribution of elevated TDG to the probability of mortal injury was minimal compared to that of LRP.

Condition factor was also found to be a statistically significant variable to help explain the variability observed in probability of mortal injury. Like TDG, condition factor contributed a very small amount to prediction of mortal injury compared to LRP. Condition factor is calculated by mass relative to body length and is indicative of fat storage or energy storage in fish (Bennett 1970). Many authors have debated the influence of the amount of fat in the body of a fish on susceptibility to injury by rapid decompression. Theoretically, fish with a greater amount of fat relative to total mass are at increased risk of barotrauma injury due to increased gas solubility in fat (St John 2003) and thereby a larger amount of gas available for change in state during rapid decompression. Specifically, the solubility coefficient for nitrogen in fat is five times larger than that of lean tissue or blood (Shilling et al. 1976). Reduced susceptibility to barotrauma for fat fish may be the result of a decrease in the amount of air needed in their swim bladder to achieve neutral buoyancy. Hypotheses for mechanistic arguments that fat fish are less susceptible to barotrauma remain untested (St John 2003). In our study, we did not quantify the amount or location of fat in the bodies of test fish. Our surrogate variable for fish fatness, condition factor, while significant in our analysis of deviance, was confounded with TDG. We conclude that condition factor has little effect on the probability of mortal injury from rapid decompression but do recommend that future research be conducted to determine the importance of fat in relation to barotrauma.

Despite the significance of condition factor in the full model predicting mortal injury, the variables used to compute condition factor—the length and weight (size) of juvenile Chinook salmon—were not significant contributors to the deviance model. This is in contrast to the hypotheses of Beyer et al. (1976) that smaller fish may be more susceptible than larger fish to barotrauma by rapid decompression because of the relatively small size of their vasculature in relation to the size of emboli. After a similar decompression scenario, Beyer et al. (1976) hypothesized that similar ranges of bubble sizes theoretically would occur in both small and large fish, assuming all sizes of fish were at equilibrium with external gas tension. They suggested that because smaller fish have smaller blood vessels, it may be more likely that they would have more severe problems from flow blockages by bubbles than would larger fish (Beyer et al. 1976). However, it is unclear if their hypothesis is consistent with the physics of bubble formation. Together, for the range of fish length and weight tested in this experiment, these variables seem to have little importance when predicting mortal injury in this application.

We hypothesized that the rate at which the pressure decreased as the fish were exposed to STP would influence mortal injury. Although ROC was a significant factor, it contributed a very small amount to prediction of mortal injury compared to LRP. It is possible that turbine passage may occur at such a high rate that fish are not always able to spit air from their swim bladder. However, rates of pressure change much higher than examined during this research have been noted at other dams within the Columbia River basin (e.g., Cougar Dam; Duncan 2010). It is unclear if these higher rates of pressure change would contribute to mortal injury more than the conditions we tested.

Histology

We also determined through histological procedures that those fish that died due to rapid decompression had a high frequency of internal injuries. These internal injuries were most often hemorrhaging of a major organ (heart, kidney, brain) or vasculature but also included intestinal eversion and gill dilation. These observations highlight the importance of using internal observations to assess the negative impacts on turbine-passed fish. Specifically, fish may appear to be healthy externally but actually have serious internal injuries. Many authors have described the presence of both internal and external barotrauma-related injuries following turbine passage (e.g., Davies 1988; Brown et al. 2009) or other sources of rapid decompression (e.g., Rummer and Bennett 2005). However, the observation of internal sources of injury and mortality are notably absent in other research articles. Given that mortality can result from internal injuries alone, we recommend that future studies examining the effects of turbine passage or other sources of rapid decompression of fish include internal observations as an endpoint. This endpoint should be coupled with external injury observations, especially considering that fish suffering from barotrauma-related injuries might not die immediately, highlighting the importance of internal observation. The mortal injury index and the methods used to build it are applicable to other injury and mortality data for juvenile salmonids from laboratory and field studies related to all dam passage routes that include collision, strike, and shear and decompression injuries (McKinstry et al. 2007).

A Comparison of Hatchery-Reared and Seaward-Migrating Sources

The comparison of juvenile Chinook salmon due to source was relatively inconclusive. Although the overall model did not show significant differences, the alpha was close to 0.05 ($P = 0.0541$). In addition, there were significant differences in the LRP coefficient between the two groups. However, there was not a difference in the intercept coefficient between the models. Due to the relatively low number of trials conducted with seaward-migrating fish, these differences in mortal injury at higher LRP could be due to limitations in sample size. Also, Sensor Fish research tends to indicate that LRP in the 1.5–2.5 range may be relatively uncommon compared to lower LRP (Carlson et al. 2008). In addition, a large portion of the seaward-migrating fish seen passing turbines at USACE dams are likely from hatchery sources (Raymond 1988). Thus, use of hatchery-reared juvenile Chinook salmon as a surrogate for seaward-migrating individuals appears to be appropriate.

Conclusions and Recommendations

This research indicates that the main factor associated with mortal injury of juvenile Chinook salmon during simulated turbine passage was the ratio between acclimation and nadir pressures. Specifically, the likelihood of mortal injury increased with higher pressure ratios. However, TDG, condition factor, and ROC contributed a very small amount to the prediction of mortal injury.

Although this research provides valuable information about the relationships between rapid decompression and barotrauma for juvenile Chinook salmon, further research is needed to determine how other species are influenced by rapid decompression associate with turbine passage. Species such as sockeye salmon (*O. nerka*), chum salmon (*O. keta*), and Atlantic salmon (*S. salar*), steelhead, and other migratory physostomous species should be studied in relation to rapid decompression. In addition, it is unclear how juvenile migratory Pacific lamprey (*Entosphenus tridentatus*) might be affected by similar

STP, given the anatomical absence of a swim bladder. However, understanding this relationship will be important, based on the Endangered Species Act listing of these and other aquatic species found in the Columbia River basin. In addition, damage due to rapid decompression during turbine passage should be assessed in migratory physoclistous species such as river herring (alewife, *Alosa pseudoharengus*, and blueback herring, *A. aestivalis*) that exist in other regions.

This research should assist engineers and fisheries managers in operating and improving hydroelectric facilities while minimizing mortality and injury of turbine-passed juvenile Chinook salmon. Using these data, models can be built that might determine how much mortal injury is present at different turbine operations as pressures change. Further, pressure data coupled with the mortal injury data should be useful to engineers and turbine manufacturers when aiming to design new turbines, which could not only increase power generation and efficiency but also minimize barotrauma as fish pass through turbines. However, these turbine designs would require knowledge of the depth to which fish are acclimated. Thus, there is also a need to better understand the depth that fish species can attain or at which they are neutrally buoyant.

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Chapter 3

Assessment of Factors Influencing Mortal Injury of Tagged Juvenile Chinook Salmon Exposed to Rapid Decompression

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Introduction

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, 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 (i.e., over spillways, juvenile bypass facilities, through turbines) and survival of the fish associated with hydropower facility passage (Bickford and Skalski 2000; Muir et al. 2001; Skalski et al. 2002).

As a way of monitoring routes of passage and survival past hydropower facilities, fish are outfitted with telemetry tags. In the Columbia and Snake rivers alone, nearly 2 million salmonids are implanted with passive integrated transponder (PIT) tags 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 tagged with telemetry tags are assumed to have rates of mortality similar to those of untagged individuals, and survival estimates are 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 passage routes through hydropower facilities of particular concern for managers and researchers is through hydro turbines (Mathur et al. 1996; Coutant and Whitney 2000; Čada et al. 2006). Passage through hydro turbines 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 (Čada 2001). Modifications to turbine design and operating conditions have been studied to limit the occurrence of these negative influences, but they have not been eliminated (Čada et al. 2006). Although the areas in which fish may be exposed to mechanical strike, cavitation, and shear forces in a hydro turbine are limited (Coutant and Whitney 2000; Neitzel et al. 2000), rapid decompression, and possibly associated barotraumas, is 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, as well as other injuries (Feathers and Knable 1983; Rummer and Bennett 2005; Brown et al. 2009).

For a fish, the regulation of gas in the swim bladder allows it 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, salmonids increase

the volume of gas in their swim bladder by gulping air at the water surface. In addition, they can expel gas from the swim bladder through their mouth to maintain buoyancy when moving up in the water column. Gases are also found in the blood and tissues of fish. At higher acclimation pressures, there is a greater mass of gas within the tissues and blood. When juvenile salmonids pass through hydro turbines, free gas in the swim bladder expands as they are exposed to rapid decompression. Simultaneously, gas dissolved in the blood and tissues come out of solution and exists as free gas in the fish's vasculature and tissues. The change in pressure can be so rapid that 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). Also, gases in the tissues and blood may come out of solution and form bubbles, or emboli, during rapid decompression (Brown et al. 2009). The formation of, and increase in size of, emboli increases the volume of the blood, thereby intravascular pressure, and can cause damage to blood vessels, resulting in hemorrhaging or entry of bubbles into organs, which may compromise their function. Other barotraumas resulting from rapid decompression can include exothalmia (eye-popping), hemorrhaging, and emboli in the fins (called emphysema), all of which have the potential to impair the fish's behavior and survival (Brown et al. 2009).

The change in pressure from acclimation to exposure, expressed as a ratio, is a large factor in predicting the likelihood of barotraumas for fish exposed to rapid decompression (Brown et al. 2009). Because fish acclimated to deeper depths have a greater mass of gas in their swim bladder, 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. The Brown et al. study, however, tested fish over only a narrow range of LRPs (natural log of the ratio between acclimation and exposure pressure—1.7 to 3.3), and the LRP experienced by fish passing through hydro turbines varies within hydropower facilities depending on acclimation depth of fish, turbine design, and operating conditions. To fully understand the influence of hydro turbine passage on fish, it is necessary to investigate the likelihood of barotraumas across a wide range of changes in pressure, representative of the occurrence at hydropower facilities.

Another factor that influences the occurrence of barotrauma in hydro turbine-passed fish is the burden associated with carrying a telemetry tag. Brown et al. (2009) showed that fish carrying a transmitter had higher rates of mortality compared to non-tagged individuals exposed to simulated turbine passage (STP). Fish implanted with telemetry tags can compensate for the additional mass of the tag by increasing their displacement 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 fish at increased risk of barotrauma and mortality when exposed to STP. Because a wide size range of juvenile salmonids are exposed to hydro turbine passage, and a wide range of tag types and sizes are used to study these fish, the influence of the tag burden (tag mass divided by fish mass) may be highly variable. However, there is a paucity of research on this subject and therefore, a need to investigate how a range of tag burdens affects the likelihood of barotrauma occurring during hydro turbine passage.

The objective of this research was to identify the factors that influence the injuries and mortality of juvenile Chinook salmon carrying a range of tag burdens and exposed to a range of pressure changes. Factors taken into account included LRP (natural log of the ratio between acclimation and nadir pressure), tag burden, tag type, fish length, fish weight, and condition factor. We hypothesized that the risk of mortality to fish bearing telemetry tags during STP would not be different 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 PNNL Aquatic Research Laboratory (ARL, in Richland, Washington; Table 3.1). All fish were either acquired as fry or hatched and reared at the ARL. While in holding, juvenile Chinook salmon were held in 1100-L circular holding tanks with flow-through ambient well water (17°C) and nourished with Bio-Diet moist pellets (Bio-Oregon, Longview, Washington) ad libitum.

Table 3.1. Median and range of fork length, mass and condition factor for each treatment group exposed to STP. Treatments represent the different transmitter types and combinations used (acoustic and PIT). This study used two variations of acoustic tags (single- or double-battery) and combinations with and without a PIT tag.

Transmitter treatment	n	Fork length (mm)	Mass (g)	Condition factor
		Median ± S.D. (Range)	Median ± S.D. (Range)	Median ± S.D. (Range)
Double battery PIT	1599	129 (95-226)	26.2 (8.1-144.7)	1.15 (0.77-2.10)
Single battery PIT	1666	133 (95-205)	29.6 (7.9-119.3)	1.17 (0.74-1.64)
Single battery only	1859	122 (83-204)	20.5 (6.5-114.6)	1.16 (0.85-1.74)
PIT only	1826	119 (79-212)	19.0 (5.8-117.1)	1.15 (0.88-1.83)
Non-tagged*	3785	124 (78-205)	20.4 (4.8-134.0)	1.10 (0.65-1.77)

* Non-tagged individuals were anesthetized as described for tagged individuals but did not undergo surgery.

Implantation of Tags

Fish were netted from a holding tank and held in a bucket containing approximately 15 L of aerated water. Each fish was then anesthetized in a tricaine methanesulfonate solution (MS-222; 80 mg/L of water), which also contained PolyAqua (0.15 mL/L of water; Kordon Aquarium Products, Hayward, California) until they reached stage 4 anesthesia (Summerfelt and Smith, 1990). While under anesthesia, fish were measured for fork length (FL; millimeters) and mass (grams). A small portion of the caudal fin was clipped for individual identification as outlined in Chapter 1.

Fish then had one of four transmitter types surgically implanted into the intraperitoneal cavity: 1) double-battery acoustic transmitter and passive integrated transponder (PIT) tag, 2) single-battery acoustic transmitter and PIT tag, 3) single-battery acoustic transmitter only, or 4) PIT tag only (Table 3.2). There was also one treatment in which fish were not implanted with a transmitter but were only anesthetized and handled as described above. The double-battery acoustic transmitter and PIT tag and PIT-tag only treatments are currently used in the Columbia and Snake rivers to monitor the passage and survival of fish through hydroelectric facilities. Fish are double–tagged, as the PIT tag 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 in Brown et al. (2006). After surgery, fish were placed in 5-L buckets containing oxygenated water to recover.

Table 3.2. Combined mass of tags (in air and water), tag volume, and median and tag burden (percentage of fish's body weight in air) associated with each transmitter treatment group exposed to simulated turbine passage.

Transmitter treatment	Tag mass in air (g)	Tag mass in water (g)	Tag volume (mL)	Tag burden (%) Median (Range)
Double battery and PIT	0.53	0.36	0.18	2.05 (0.37-6.62)
Single battery and 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)

Acclimation Prior to Pressure Exposure and Simulated Turbine Passage

After fish recovered from surgery, denoted by regaining equilibrium, they were loaded in the hyper/hypobaric chambers of the Mobile Aquatic Barotrauma Laboratory for acclimation to 15-ft depth (21.2 psia). Fish were held for a 16–24 h period, according to the methods outlined in Chapter 1, to allow for ample time to attain neutral buoyancy and equilibration of gas tensions in bodily fluids and tissues. Buoyancy following this period was determined using the same observational methods described in earlier chapters. Although we tested more than 10735 fish, a small proportion of these fish (4% were negatively buoyant and <0.1% were positively buoyant) never gained neutral buoyancy following 16 h of acclimation. Given the results presented in Chapter 1 and the assumption that in-river fish are neutrally buoyant when approaching hydroelectric facilities (due to energy conservation in wild systems), we included only neutrally buoyant fish for the analyses presented in this chapter.

Water supply to the chambers was consistent with that outlined in Chapter 2. Ambient well water was supplied to the chambers (median temperature = 17.0°C; range = 15.4°C to 17.9°C) and total dissolved gas (TDG) levels were about 115% saturation (median = 115.0%; range 112.7% to 127.5%).

Exposure Pressures and Rate of Pressure Change

Exposure pressures (nadir pressures) ranged from 1.3 to 29.1 psia (median = 6.8 psia), and the rate of change during STP ranged from 15 to 555 psia/s (median = 217 psia/s).

Fish Removal and Necropsy

After STP exposure, fish were euthanized with an overdose of MS-222 (250 mg/L of water). Necropsies were performed as detailed in Chapter 1. Mortal injury served as the endpoint of all analysis (as detailed in Chapter 2).

Statistics

The experimental factors examined in this study were 1) natural log of the ratio between acclimation and nadir pressure (LRP; as detailed in Chapter 2), 2) tag burden, 3) transmitter treatment, 4) condition factor (as detailed in Chapter 2), 5) fish length, and 6) fish weight. In total, 10742 fish were surgically

implanted with one of five transmitter combinations (double-battery acoustic tag and PIT tag, single-battery acoustic tag and PIT tag, single-battery acoustic tag only, PIT tag only, or no tag) and then either exposed or not to STP in a replicated experimental design. Fish were then examined externally, followed by necropsy for the presence of barotraumas, specifically those that have been identified as mortal injuries. Data were used in the subsequent analysis only if nadir pressures were equal to or lower than 29.1 psia during simulated turbine passage.

Statistical Models

Mortal injury was modeled 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

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

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 using a measure called area under the curve (AUC; Hosmer and Lemeshow 1999). 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 or stochastic 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 3.1), which plots true positive rate versus false positive rate. We compared AUC values between alternative logistic models to identify their relative levels of performance. The AUC value is the area under the curve inside the unit square.
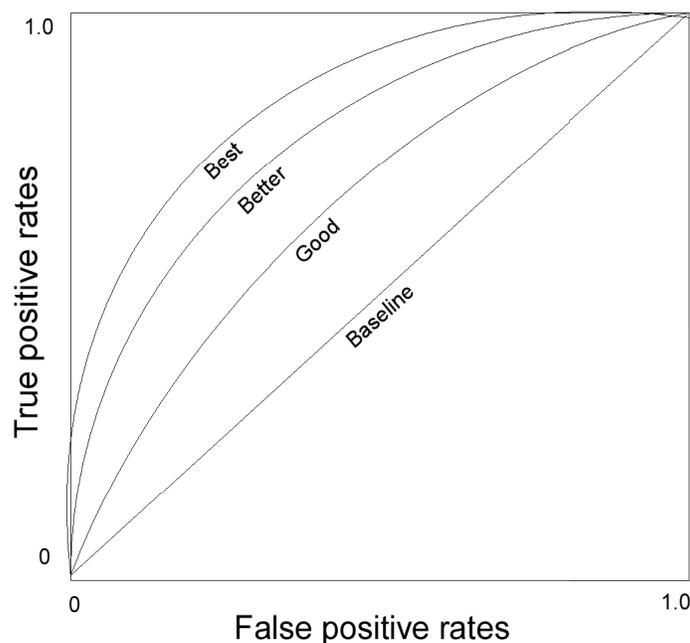

Figure 3.1. Area under the curve with alternative model performance curves.

Results

Factors Associated with Mortal Injury

The main treatment factors of LRP (P < 0.0001), tag burden (P < 0.0001), fish length (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 3.3). Fish weight was not significant (P = 0.2367). The main model plus the seven interactions explained 44.4% 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 in the model. The other variables and interaction factors combined explained less than 1% of the variability in the model (fish length = 0.3%, tag type = 0.1%, condition factor = 0.06%, fish length × tag type = 0.2%, fish length × condition factor = 0.07%, LRP × tag burden = 0.006%, LRP × fish length = 0.006%, LRP × tag type = 0.1%, tag burden × tag type = 0.05%, and LRP × condition factor = 0.03%).

Similar to the analysis of deviance results, the area under the curve (AUC) analysis also indicated 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 3.2). The model containing only LRP explained a large portion of the variability in the model with an AUC score of 0.8783. However, the model containing LRP and tag burden had an AUC score of 0.9034, thus explaining all significant variability in the model. This simplified model using LRP and tag burden appears to be an adequate predictor of mortal injury.

Table 3.3. Sequential analysis of deviance of the factors associated with the mortal injury of tagged juvenile Chinook salmon with respect to simulated turbine passage. The final model explained 42.1% of the variability in the data; see text for details. Factors include LRP = natural log of the ratio of acclimation to exposure pressure, TB = tag burden, L = fish length (mm), TT = tag type, and CF = condition factor. Factors right of the "|" symbol indicate that those factors were already in the model when the factor to the left was examined.

Source	df	Deviance	Mean deviance	F	P
Total$_{cor}$	10741	14694.2			
Main Effects					
LRP	1	5237.2	5237.2	5947.8	< 0.0001
TB | LRP	1	930.3	930.3	1171.7	< 0.0001
L | 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 × TT | LRP + TB + L + TT + CF	4	32.4	8.1	10.3	< 0.0001
L × CF | LRP + TB + L + TT + CF + L × TT	1	29.8	29.8	38.1	< 0.0001
LRP × TB | LRP + TB + L + TT + CF + L × TT + L × CF	1	20.7	20.7	26.5	< 0.0001
LRP × L | LRP + TB + L + TT + CF + L × TT + L × CF + LRP × TB	1	20.7	20.7	26.6	< 0.0001
LRP × TT | LRP + TB + L + TT + CF + L × TT + L × CF + LRP × TB + LRP × L	4	15	3.7	4.8	0.0007
TB × TT | LRP + TB + L + TT + CF + L × TT + L × CF + LRP × TB + LRP × L + LRP × TT	3	7.8	2.6	3.3	0.0195
LRP × CF | LRP + TB + L + TT + CF + L × TT + L × CF + LRP × TB + LRP × L + LRP × TT + TB × TT	1	4.2	4.2	5.3	0.0213
Error	10718	8320.5	0.78		
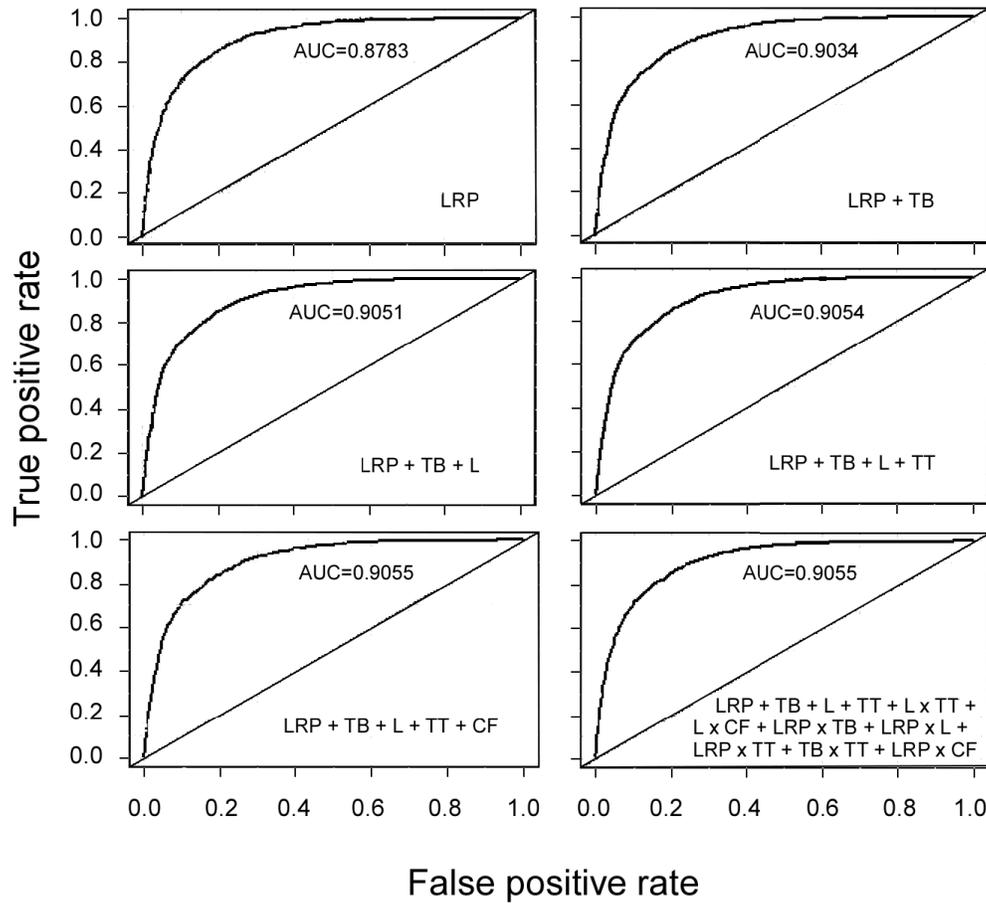
Figure 3.2. Area-under-the-curve (AUC) plots of the predictive value from each progressive model and the actual mortal injury outcome. Factors include LRP = natural log of the ratio between acclimation and exposure pressure, TB = tag burden, L = fish length, TT = tag type and CF = condition factor.

An analysis of deviance of this simplified model indicates that LRP and tag burden are significant predictors of mortal injury ($P < 0.0001$ and $P < 0.0001$, respectively; Table 3.3), explaining 41.9% of the variability in the model. Equation (3.1) for predicting mortal injury given LRP and tag burden (%) is

$$\text{Probability of mortal injury} = \frac{e^{-5.997 + 4.201*\text{LRP} + 0.603*\text{Tag burden}}}{1 + e^{-5.997 + 4.201*\text{LRP} + 0.603*\text{Tag burden}}}$$

(3.1)

Using the data collected in this study, three-dimensional plots were constructed to display the relationship among LRP, tag burden, and mortal injury (Figure 3.3). These plots demonstrate that as LRP and tag burden increase, mortal injury also increases.
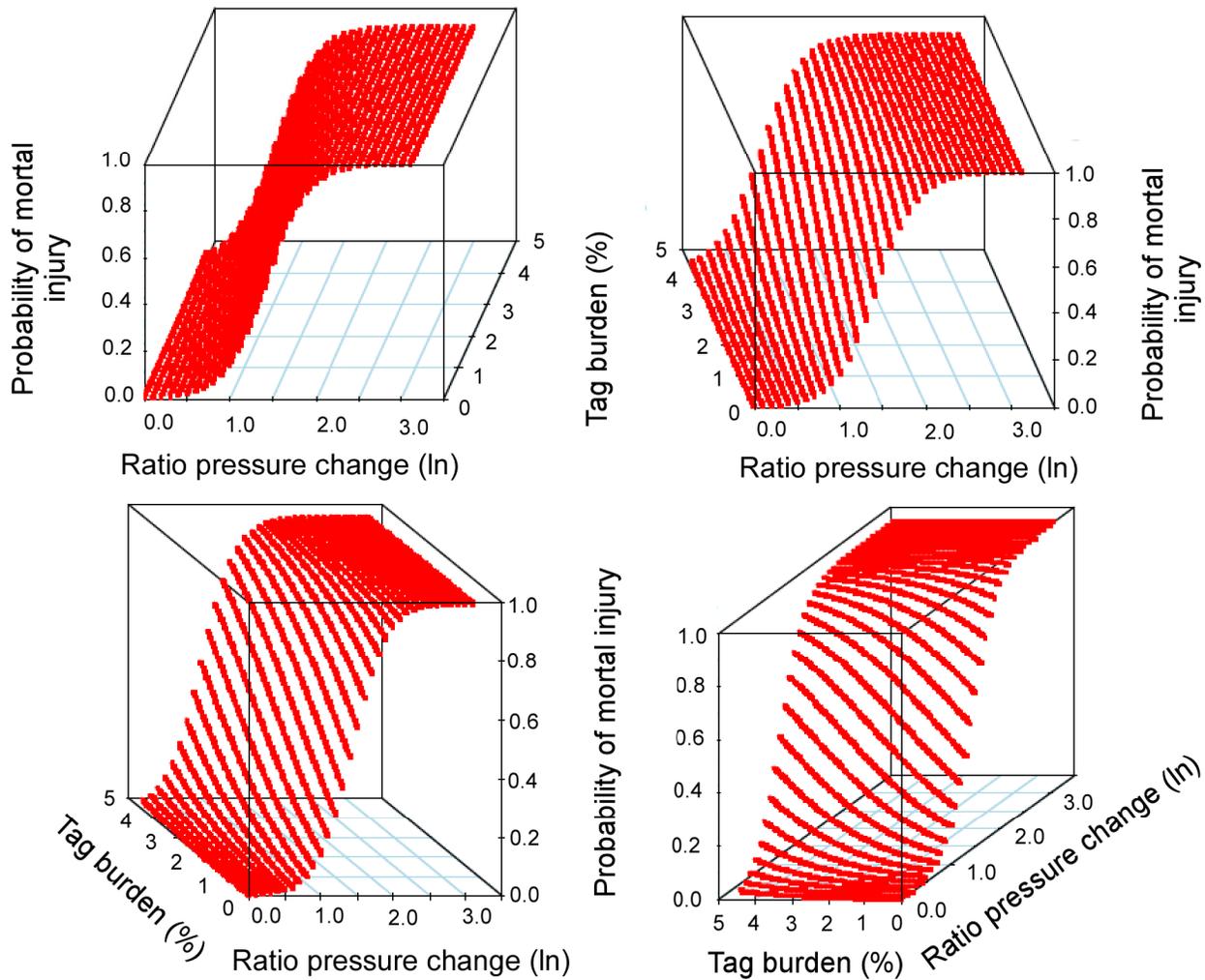
Figure 3.3. Overlays of fitted logistic curves of mortal injury along a range of LRP (ratio of pressure with natural log applied) and tag burden for juvenile Chinook salmon. Plots were rotated on the *z*-axis of mortal injury to provide alternative perspectives of the surrounding plane. In the current study, tag burdens of 0.0% to 6.6% were tested.

Tag Expulsion

Throughout the study, three acoustic tags and six PIT tags were expelled (through the surgical incision) during STP exposure (Table 3.4). Acoustic tags were expelled over varying LRP values (0.95, 2.22, and 2.74); the double-battery acoustic tag was expelled at the lowest LRP (0.95). PIT tags were also expelled over a range of LRP (Table 3.4; mean = 1.64). Most (83.3%) of the PIT tags expelled (5 of 6) were from fish also implanted with an acoustic tag. None of the fish that expelled tags died during or shortly after exposure to STP; however, 100% (3 of 3) of fish that expelled acoustic tags were mortally injured, and 83.3% (5 of 6) fish which expelled PIT tags were mortally injured as a result of STP exposure.

Table 3.4. Number of telemetry tags expelled during simulated turbine passage and corresponding LRP (natural log of the ratio between acclimation and nadir pressures) values.

Treatment type		Acoustic tags dropped				PIT tags dropped		
	n	n	%	LRP		n	%	LRP
Double battery and PIT	1599	1	0.10%	0.95		2	0.10%	1.38, 1.53
Single battery and PIT	1666	1	0.10%	2.22*		3	0.20%	0.86, 2.22*, 2.43
Single battery only	1859	1	0.10%	2.74		0	0.00%	NA
PIT tag	1826	0	0.00%	NA		1	0.10%	1.4

* Fish expelled both acoustic and PIT tag.

Discussion

Factors Associated with Mortal Injury

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

Changes in pressure have previously been shown to be 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; Chapter 2). However, Brown et al. (2009) examined the occurrence of injury and mortality for a range of LRP from only 1.7 to 3.3 (ratios of pressure change from 5.3 to 27.0), while this current study examines the likelihood of mortal injury from no pressure change to an LRP of 3.12 (ratios of pressure change from 0.0 to 22.6) (Figure 3.4). Only the current study and the work done by Brown et al. (2009) have addressed the additional influence of a telemetry tag on barotrauma due to rapid decompression.

This research has indicated that, as tag burden increased, the rate of mortal injury also increased. The presence of a telemetry tag has also been shown to influence injury and mortality rates by other researchers (Brown et al. 2009). Brown et al. (2009) examined the effects of rapid decompression associated with the presence of a telemetry tag (with tag burdens ranging from 1.3% to 4.7%) for juvenile Chinook salmon. Smaller fish (subyearlings) acquired higher rates of injury and mortality associated with STP when compared to larger individuals (yearlings). However this could not be clearly linked to the tag burden carried by smaller fish, as tag burden was similar for both subyearling and yearling fish (subyearling: mean = 2.9%, range = 1.4% to 4.2%; yearling: mean = 3.1%, range = 1.3% to 4.7%; Brown et al. 2009). The current work examined a broader range of tag burdens (0.0% to 6.6%) and looked specifically at the relationship between tag burden and the likelihood of mortal injury. The current study also expanded the relationship between tag burden and mortal injury over a range of LRPs. The highest

rates of mortal injury were seen for fish exposed to high changes in pressure and carrying high tag burdens. By comparison, fish exposed to low changes in pressure and carrying low tag burdens exhibited lower rates of mortal injury. The understanding of this relationship is important for application to field studies examining turbine survival through hydropower facilities.
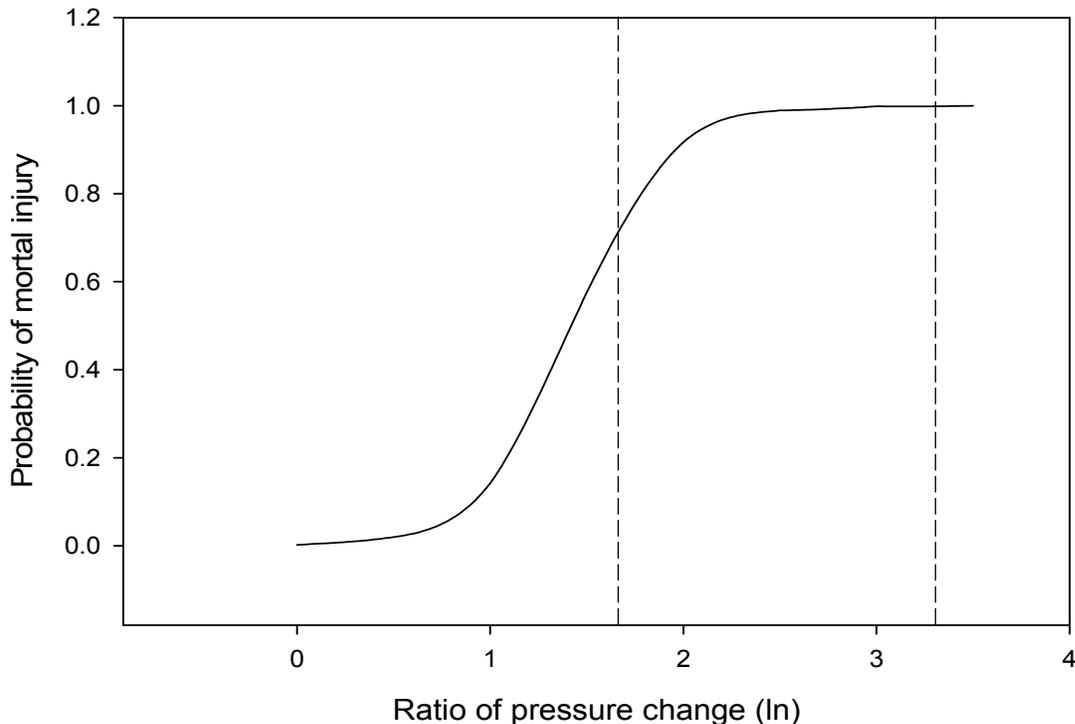

Figure 3.4. Probability of mortal injury for an untagged fish experiencing a range LRPs based on current study results. Previous research (Brown et al. 2009; area between dotted lines) only examined injury and mortality related to LRP values of 1.7 to 3.3, while the range of LRP in the current study was from no pressure change to 3.12.

Tag burden has varied considerably among field studies conducted within the Columbia and Snake rivers examining route-specific survival, including through hydro turbines. Fish have been tagged with a variety of tag types, including radio, acoustic and PIT tags, which have varied in mass from 0.1 g (for an implanted PIT tag; e.g., Achord et al. 2009) to 1.4 g or higher (combined weight of implanted radio and PIT tags; e.g., Hockersmith et al. 2003). The size of fish being used to examine hydro turbine passage survival at USACE projects in the Snake and Columbia rivers also varies. The range of fish size used is difficult to discern, as many reports do not state minimum and maximum sizes of fish. Based on information available in reports, it can be estimated that tag burdens of juvenile Chinook salmon used in these studies generally ranged from 1.0% to 6.4% (although tag burdens for some studies were not reported), depending on size of fish and tag type used (Table 3.5). Because many reports provide only the fish length, the mass of the fish for these other studies was estimated from length–weight relationships collected by the authors of the current study to estimate tag burden. Increased rates of injury and mortality resulting from these tag burdens used in these studies may have created bias in survival estimates.

Table 3.5. Summary of fish life stage and size, tag type, tag mass, and tag burden used in survival estimates of juvenile Chinook salmon passing through hydro turbines in the lower Columbia River. Studies using gastrically implanted transmitters were excluded. *Fish size was reported as > 95 mm, and the fish mass was calculated using a length–weight relationship for Chinook salmon by the authors of this report. Survival estimates reported in the table come from the corresponding adjacent reference.

Dam	Life Stage	Tag Types	Tag Mass (g)	Fish Mass (g)	Tag Burden (%)	Survival estimate (%)	Reference
John Day							
	Subyearling	JSATS + PIT	0.525	median = 20.0	median = 2.63	72.8	Weiland et al. 2009
	Yearling	JSATS + PIT	0.585	median = 40.0	median = 1.46	85.5	Weiland et al. 2009
Bonneville							
	Subyearling	Acoustic + PIT	0.525	median = 20.0	median = 2.63	93.3	Faber et al. 2010
	Yearling	Acoustic + PIT	0.525	median = 40.0	median = 1.31	94.8	Faber et al. 2010
	Subyearling	JSATS + PIT	0.525	>9.2	< 5.7*	93.3	Ploskey et al. 2009
	Yearling	JSATS + PIT	0.585	>9.2	< 6.4*	94.8	Ploskey et al. 2009

To illustrate the interaction between tag burden and pressure change on mortal injury, the estimated probability of mortal injury for fish with a broad range of tag burdens representative of in-river studies was determined using Equation (3.1) (Figure 3.5). The difference in mortal injury among tag types was low when pressure changes were 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 among fish with higher tag burden. At very high pressure changes, 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.
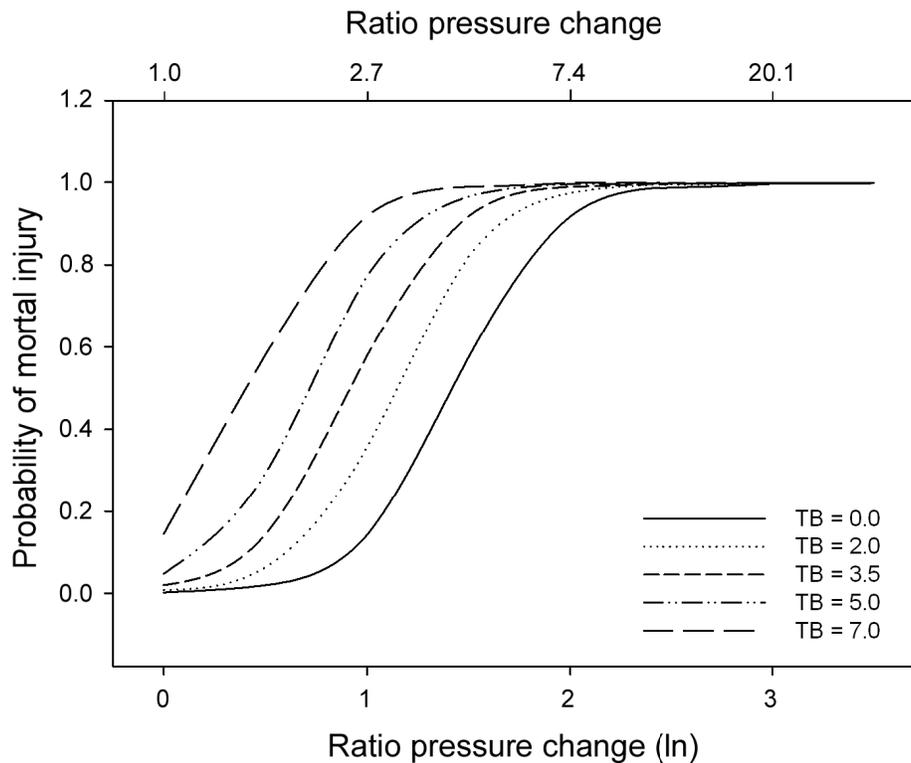
Figure 3.5. Comparison of probability of mortal injury associated with different tag burdens for juvenile Chinook salmon. In the current study, tag burdens of 0.0% to 6.6% were tested. TB = tag burden (%).

Equation (3.1) is appropriate for estimating mortal injury for tagged fish compared to untagged fish. However, we recommend using Equation (2.6) for applications such as guidance of design for new turbines. There is very little difference (~1% or less along the entire range of pressure change) in the relationship between mortal injury and pressure change for untagged fish using either equation. However, fish tested to derive Equation (2.6) were exposed to a wider range of TDG and acclimation pressures than those tested to derive Equation (3.1). In addition, the samples sizes of untagged fish used to derive Equation (2.6) are higher than the sample sizes of untagged fish used to derive Equation (3.1). Therefore, before using these equations, researchers should consider the objective of their investigations to ensure the appropriate equation is used.

Based on the results of the current study, the tag burden of implanted fish used in survival studies (Table 3.5) may have influenced the reported survival estimates of turbine passed juvenile Chinook salmon. Survival estimates for turbine passed fish at three hydropower facilities in the Columbia River range from 67.0% to 99.3%, with estimates generally lower for subyearling juvenile Chinook salmon compared to yearlings (Table 3.6). To illustrate the possible bias due to carrying a telemetry tag during turbine passage, we use the results from Weiland et al. (2009) as an example. Weiland et al. (2009) reported that survival of subyearling Chinook salmon (median mass = 20.0 g; median tag burden 2.63%; Table 3.5) passing through hydro turbines at John Day Dam was 72.8% (Table 3.5). Using Equation (3.1), we can estimate that fish with this tag burden would be likely to experience this survival rate if they were acclimated to 10 ft of depth (19.0 psia) and exposed to a pressure nadir of 8.4 psia during turbine passage (a LRP of 0.82 or a ratio of pressure change of 2.3). Using Equation (3.1), we estimate that untagged fish exposed to the same conditions would have a survival rate of 92.9% (20% higher than tagged fish). When this bias in turbine survival is applied to overall dam passage survival (concrete passage), the estimate increases by 3.3% from the 86.1% that Weiland et al. (2009) reported to 89.4%.

However, the bias due to tagging would be much lower for fish with a lower tag burden. For example, Weiland et al. (2009) reported that survival of yearling Chinook salmon (median mass = 40.0 g; median tag burden 1.46%; Table 3.5) passing through hydro turbines at John Day Dam was 85.5% (Table 3.5). Using Equation (3.1), we can estimate that fish with this tag burden would be likely to experience this survival rate if they were acclimated to 10 ft of depth (19.0 psia) and exposed to a pressure nadir of 8.6 psia during turbine passage (a LRP of 0.80 or a ratio of pressure change of 2.2). Using Equation (3.1), we estimate that untagged fish exposed to the same conditions would have a survival rate of 93.4% (7.9% higher than tagged fish). When this bias in turbine survival is applied to overall dam passage survival (concrete passage), the estimate increases by 0.6% from the 95.8% that Weiland et al. (2009) reported to 96.4%.

The examples above, which are not a complete assessment of the risk of mortal injury for a population of turbine passed fish, illustrate how important tag burden is when estimating survival of turbine passed fish. Some researchers have recommended tag burden should be no higher than 2% of the fish's total mass, (Winter 1996). This general "rule" has been questioned by several authors (Brown et al. 1999, 2010; Jepsen et al. 2002; Jepsen 2003) who have found some aspects of fish fitness not to be influenced by higher tag burdens (e.g., growth, survival, swimming performance). 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 suggest that more accurate estimates of hydro turbine passage survival are likely to be attained by using the smallest possible telemetry tag.

Several variables other than LRP and tag burden were 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, there are several types of telemetry tags 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. However, this importance is minute compared to the LRP and tag burden. Several researchers have examined the importance of tag shape among externally attached tags, due to the potential for creating drag while the fish is swimming, as well as the potential for snagging 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 the topic may be equally important, there is a paucity of information on the influence of tag shape on fish surgically implanted with telemetry tags. 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, resulting in puncture and compression injuries. Although the current study used telemetry tags of similar shapes, a focus for future research should examine the influence of differences in tag shape on the likelihood of mortal injury for hydro turbine-passed fish.

Table 3.6. Survival estimates of juvenile Chinook salmon passing through turbines at one of three hydropower facilities found in the Columbia River. The table illustrates the project-specific variation in annual survival estimates using tagged juvenile Chinooks salmon.

Year		Bonneville Dam Powerhouse 2	The Dalles Dam	John Day Dam
	Yearling			
2000			81.0 [1,2]	
2002		99.3 [1,5]	85.0 [3]	77.8, 83.2 [2,4]
2003				76.4, 82.0 [1,3]
2004		95.1 [2]	80.0 [4]	
2005		96.6 [3]	84.0 [5]	
2008		94.8 [4,6]		85.5, 84.4 [5]
2009				
Mean		96.5	82.5	81.6
	Subyearling			
2000			84.0 [1,2]	
2002			82.0 [3]	86.6, 96.6 [2,4]
2003				71.9, 72.2 [1,3]
2004		82.5, 83.3 [2]	67.0 [4]	
2005		89.5 [3]	80.0 [5]	
2008		93.3 [4,6]		72.9, 74.9 [5]
2009				
Mean		87.2	78.3	78.6

Bonneville Dam: Counihan et al. 2003[1]; Counihan et al. 2006a[2]; Counihan et al. 2006b[3]; Faber et al. 2010[4]; Evans et al. 2003[5;] Ploskey et al. 2009[6].
The Dalles Dam: Absolon et al 2002[1]; Beeman et al. 2005[2]; Counihan et al. 2006d[3]; Counihan et al. 2006f[4]; Counihan et al. 2006g[5].
John Day Dam: Beeman et al. 2005[1]; Counihan et al. 2006c[2]; Counihan et al. 2006e[3]; Hansel et al. 2004[4]; Weiland 2009[5].

Although it is likely highly correlated with the mass of the tag, the volume that the tag occupies may also be an important parameter in relation to barotrauma. During rapid decompression, the expansion of gases in the swim bladder and tissues may reduce the available volume of space within the intraperitoneal cavity, which is finite, where the tag rests. The presence of a tag may limit the volume that the gases can expand to before barotraumas such as compression-related injuries occur. It is possible that two tags of equal mass but different volume could have different barotrauma response, particularly if the fish had to hold the same mass of gas in its 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 hydro turbine–passed fish. Brown et al. (2009) reported that gastrically tagged juvenile Chinook salmon were more likely than surgically implanted or untagged salmon to be injured or die as a result of STP. It was hypothesized that this was in part influenced by the incision through the body wall of the fish. This incision was not fully healed during STP exposure and so may have promoted stretching of the body wall to permit the release of gases, thus possibly decreasing barotraumas. For the current study, all fish implanted with tags had some kind of vent to the intraperitoneal cavity (puncture from PIT tag insertion or surgical incision). While mortal injury did not differ by tag type (thus PIT vs. surgical implantation) in this 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 hydro turbine passed fish, and thus worthy of future research.

The condition factor of fish and their length were also statistically significant variables explaining the variability in observations of mortal injury, but, like tag type, they contributed very little to the predictions of mortal injury compared to LRP and tag burden. The relationship between condition factor or fish length and barotrauma is discussed in Chapter 2. Fish weight, although included in the calculation of condition factor, was not a statistically significant variable explaining the variability in observations of mortal injury. This may be due to the high correlation with tag burden, which was a much more powerful predictor of mortal injury.

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 fish tested in this study, with only 0.1% (8 of 6950) of tagged fish expelling tags (Table 3.4). Brown et al. (2009) observed a rate of 3.1% (5 of 163) tag expulsion among gastrically tagged (with radio transmitters) juvenile Chinook salmon exposed to STP, but they reported no expulsion among fish surgically implanted with radio transmitters (1.3% to 4.7% tag burden for both implantation methods).

The number of sutures and type of knots used when incisions are closed could influence tag retention during hydro turbine passage. This research was conducted with two sutures used to close the incision (using methods described in Deters et al. 2010). However, other researchers have also used two sutures to close incisions that are almost twice the size of those made for this research (11- to 12-mm incisions were made by Welch et al.(2007) for implantation of a 1.4-g acoustic transmitter and a 0.1-g PIT tag). Future research is needed to determine the proper number of sutures needed and how suturing technique may influence tag expulsion during fish passage through hydro turbines.[1]

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[1] Boyd JW, KA Deters, and RS Brown. *Performance Assessment of Suture Number in Juvenile Chinook Salmon Implanted with Acoustic Transmitters, Following Simulated Turbine Passage.* Unpublished 2010 draft report, Pacific Northwest National Laboratory, Richland, Washington.

Conclusion and Recommendations

This research demonstrates that the derived variables—natural log of the ratio between acclimation and nadir pressures (LRP) and tag burden—are the most important factors in determining the likelihood of mortal injury for juvenile Chinook salmon exposed to rapid decompression associated with turbine passage. This research has indicated that, as tag burden increased, the rate of mortal injury also increased. Based on the results of the current study, the tag burden of implanted fish used in survival studies may have influenced the reported survival estimates of turbine-passed juvenile Chinook salmon. This, in turn can influence overall dam passage survival estimates.

The results of this study have large implications for the management of hydroelectric facilities. Findings indicate that the presence of a telemetry tag likely creates a bias in the estimates of survival for juvenile Chinook salmon undergoing rapid decompression associated with hydro turbine passage. This is especially true for those fish undergoing large changes from acclimation to nadir pressures and 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 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 hydro turbine passage survival.

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