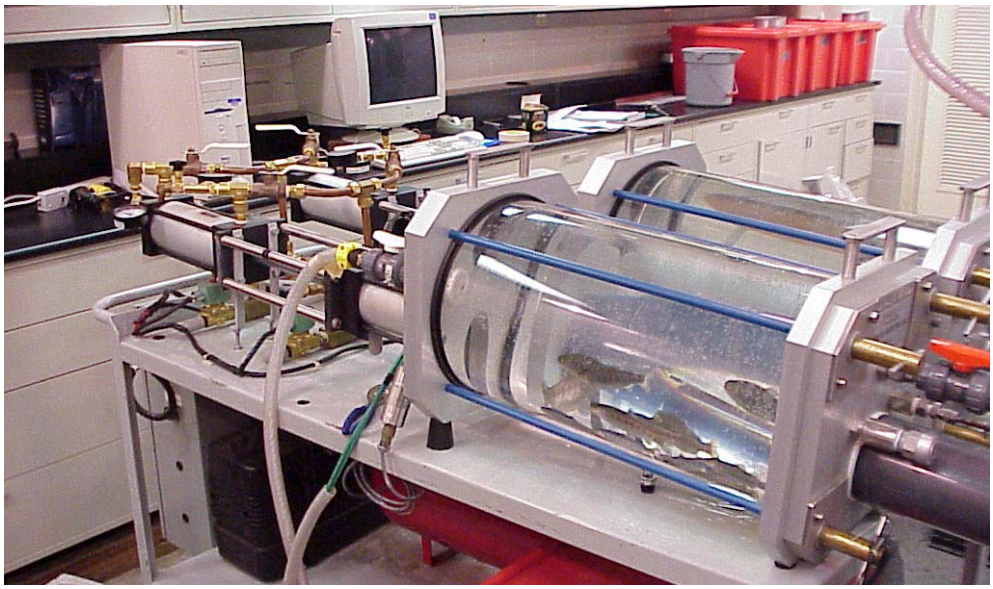
PNNL-16790

Assessment of Barotrauma Resulting from Rapid Decompression of Depth-Acclimated Juvenile Chinook Salmon Bearing Radio Telemetry Transmitters



R.S. Brown
T.J. Carlson
A.E. Welch
J.R. Stephenson
C.S. Abernethy
C.A. McKinstry
M.H. Theriault

Final Report
September 2007

Prepared for the
U.S. Army Corps of Engineers
under a Related Services Agreement
with the U.S. Department of Energy
Contract DE-AC05-76RLO1830

Pacific Northwest
National Laboratory
Operated by Battelle for the
U.S. Department of Energy

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Battelle–Pacific Northwest Division
Richland, Washington 99352

Executive Summary

A multifactor study was conducted by Battelle–Pacific Northwest Division for the U.S. Army Corps of Engineers (USACE) in 2005 to assess the significance of the presence of a radio telemetry transmitter on the effects of rapid decompression from simulated hydro turbine passage on depth-acclimated juvenile run-of-the-river Chinook salmon. Study factors were

(1) Juvenile Chinook salmon age (subyearling or yearling)

(2) Radio transmitter (present or absent)

(3) Three transmitter implantation factors (gastric, surgical, and no transmitter)

(4) Four acclimation depth factors (1-, 10-, 20-, and 40-foot submergence equivalent absolute pressure).

There were 48 unique treatments based on combinations of factors. Exposed fish were examined for changes in behavior, presence or absence of barotrauma injuries, and immediate or delayed mortality. Logistic models were used to test hypotheses that addressed study objectives.

The presence of a radio transmitter significantly increased the risk of barotrauma injury and mortality at exposure to rapid decompression. Gastric implantation presented a higher risk than surgical implantation. Fish were exposed within 48 hours of transmitter implantation so surgical incisions were not completely healed. The difference in results obtained for gastric and surgical implantation methods may be the result of study design and the results may have been different if fish tested had completely healed surgical wounds. However, the test did simulate the typical surgical-release time frame for in-river telemetry studies of fish survival so the results are probably representative of fish passing through a turbine shortly after release into the river.

The finding of a significant difference in response to rapid decompression between fish bearing radio transmitters and those not implies a bias may exist in estimates of turbine passage survival obtained using radio telemetry. However, the rapid decompression (simulated turbine passage) conditions used for the study represented near worst case exposure for fish passing through turbines. At this time, insufficient data exist on the distribution of river-run fish entering turbines, and particularly the distribution of fish passing through turbine runners, to extrapolate study findings to the population of fish passing through the turbines of the dams in the Federal Columbia River Power System.

This study is the first study examining rapid decompression that includes acclimation depth as an experimental factor for physostomous fish. We found that fish acclimated to deeper depth were significantly more vulnerable to barotrauma injury and death. Insufficient information about the distribution of fish entering turbines and their depth acclimation currently exists to extrapolate these findings to the population of fish passing through turbines. However, the risk of barotrauma for turbine-passed fish could be particularly high for subyearling Chinook salmon, which migrate downstream at deeper depths late in the early summer portion of the outmigration.

The causes of immediate and delayed mortality from rapid decompression were identified as hemorrhaging and emboli in major organs and tissues. A very frequent mechanism for injury leading to

death was the formation of bubbles and the occurrence of high blood pressure caused by dissolved gas leaving solution as blood became supersaturated in response to decreases in ambient pressure. A second major mechanism for serious injury was compression of organs caused by swimbladder expansion during decreases in ambient pressure.

Non-lethal injuries were observed that could result in behaviors that might expose turbine-passed fish to increased risk of predation by birds and other fish. The two most important were loss of equilibrium and either or both swimbladder rupture and burping of swimbladder air. For the majority of fish that showed loss of equilibrium following rapid decompression, the condition persisted for at least two hours. Loss of equilibrium results in aberrant swimming behavior and most likely other motor and sensory impairment that probably reduces the ability of fish experiencing this condition to avoid predation. Burping of air from the swim bladder during decompression and resulting negative buoyancy would likely motivate these fish to move to the surface to refill their swimbladders where they would experience increased exposure to predation. Potentially more serious is swimbladder rupture where persistent negative or positive buoyancy might result. Persistent negative buoyancy is one of the two possible consequences of a ruptured swimbladder where the rupture prevents the fish from filling its swim bladder to recover buoyancy control. Persistent positive buoyancy is a condition created when air from a ruptured swim bladder is retained within the abdomen of a fish. Air entrained in the body cavity cannot be expelled and causes the fish to lose control of buoyancy. Any persistent condition leading to loss of control of buoyancy would likely have consequences for general fitness as well as increased risk of predation.

Information about the distribution of fish entering turbines, the probability of exposure to low pressure nadirs, and point estimates of the probability of barotrauma from rapid decompression for exposure of depth-acclimated fish to pressure nadirs in the range of 1 to 14 psia is needed to complete an assessment for guidance of turbine design and operation to manage the risk of barotrauma injury to juvenile salmonids passing through turbines in the Federal Columbia River Power System.

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The authors acknowledge the support and oversight of Mike Langeslay, the contracting officer's representative for the USACE. Additional technical guidance was provided by the USACE Turbine Survival Technical Team and in particular Martin Ahmann and Dan Feil. John Skalski and Richard Townsend of the University of Washington provided valuable assistance with study design and data analysis. Ralph Elston of Aqua Technics provided the histology.

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1.0 Introduction

As a part of ongoing Federal Columbia River Power System configuration assessment, the U.S. Army Corps of Engineers (USACE) is conducting studies to identify the hydro system configuration and operations that will optimize the survival of juvenile salmonid migrants passing through the Federal Columbia River Power System. Elements of this assessment include identification of hydroturbine designs and operations to optimize the survival of juvenile salmonids passing through turbines and investigation of the use of telemetry to estimate turbine passage survival. During hydroturbine passage, juvenile fish are exposed to a number of physical phenomena that can cause injury or death; one of these is a rapid change in pressure during passage through a turbine's runner. The goal of this study, conducted by Battelle–Pacific Northwest Division for the USACE in 2005, was to investigate the impact of the presence of a radio telemetry device on the health and survival of run-of-the-river juvenile Chinook salmon exposed to worst-case simulated turbine passage pressure, factored by juvenile Chinook age class, transmitter implantation method, and depth-equivalent pressure acclimation.

1.1 Background

It is generally known that fish passing through a hydroturbine may experience rapid decompression to low pressures that can cause barotrauma resulting in injury or death (Cramer and Oligher 1964). The pressure environment of a hydroturbine is very complex and the possible number of trajectories that a fish may take through a large Columbia River mainstem Kaplan hydroturbine is very large. The result is that essentially every fish passing through a hydroturbine has a largely unique pressure exposure history, which may or may not expose the fish to low pressure (i.e., pressures between vapor pressure and atmospheric pressure, approximately 0 to 14.7 psia). Each pressure time history is bounded by the lowest pressure and highest pressure that may exist in a particular turbine, and each pressure exposure history shares a common temporal pattern resulting from passage through essentially discrete turbine regions such as the turbine intake, runner, and draft tube. The bounds on absolute pressure that a fish may experience during turbine passage are determined by the head across the turbine, the turbine's design, and the way it is being operated (USACE 2004).

Figure 1.1 is a cross section through a Kaplan turbine showing the distribution in pressure through the turbine's runner. This is a slice through the runner environment; other slices at different radial distances along the runner would show a different gradient in pressure from the suction (low pressure) side of the runner blades to the pressure (high pressure) side of the turbine blades. Fish can enter the turbine runner environment at any location in the two-dimensional horizontal plane above the runner. This plane is donut shaped with the inner diameter equal to that of the runner hub and the outer diameter equal to the diameter of the turbine discharge ring. This entry point will, in large part, predetermine the trajectory of the fish through the runner because of the high velocity (on the order of 15 m/s) and very short time of transit (on the order of 100 ms) of water through the runner. Trajectories along the pressure side of the runner blades do not expose fish to low pressures, while trajectories along the suction side do. In the space between turbine blades there is a gradient from the high pressures on the pressure side of a preceding blade to the low pressure on the suction side of a following blade. Runner passage trajectories take fish somewhere between these two extremes. Whether or not a fish is exposed to very low pressure

is a matter of chance where the risk of low pressure exposure is, in general, somewhat higher for passage near the tip and hub of runner blades for any particular operation.

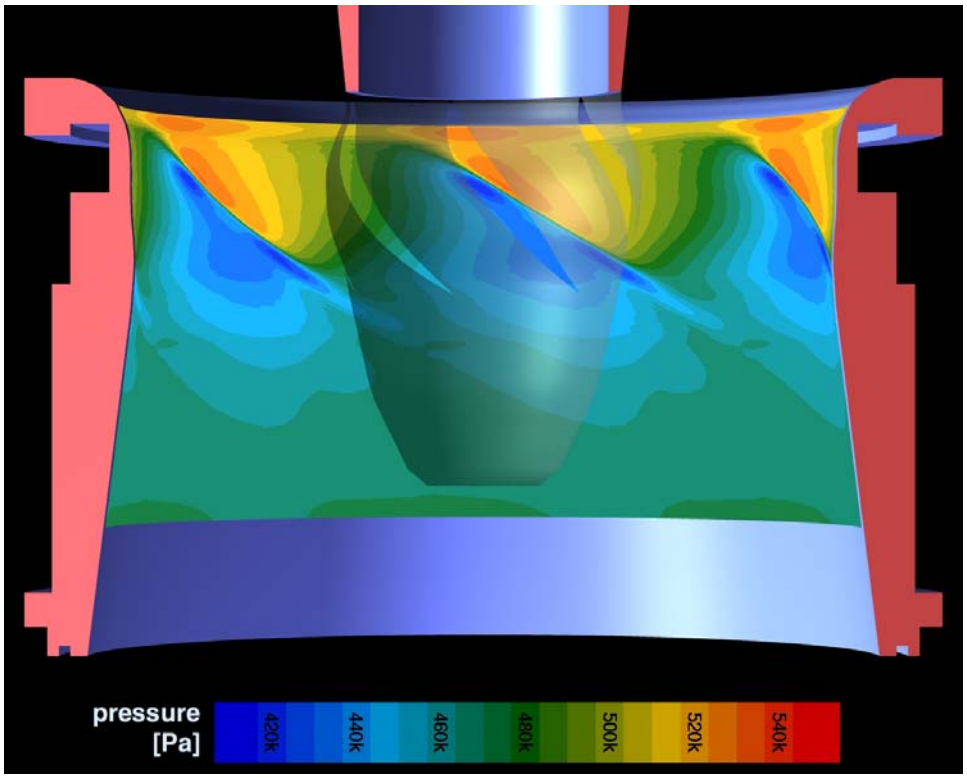


Figure 1.1. Cross Section through the Runner Region of a Kaplan Turbine Showing Pressure Distribution Simulated Using Computational Fluid Dynamics. © ETH Zürich and Escher Wyss, Visualization by ETH Zürich (R. Peikert, M. Roth), CFD Simulation by Escher Wyss, Zürich, Switzerland. Used with permission.

Biological Index Testing (BIT) is a strategy developed by the USACE Turbine Survival Program technical team to utilize information from physical turbine models, field sampling using live test fish, and studies using the Sensor Fish device to identify turbine designs and operations that optimize the passage of juvenile salmonids through turbines in the FCRPS. Among other information, implementation of BIT requires knowledge of the design and operation of turbines and the operation-dependent pressure environment in an operating turbine, and biological information about the response of fish to changes in pressure experienced during turbine passage.

The biological consequences to fish from exposure to pressure changes during turbine passage have been of considerable interest since barotrauma injuries were first observed in turbine-passed fish (Cramer and Oligher 1964). It is clear from barotrauma literature that the occurrence and severity of barotrauma injury or mortality for passage through a hydroturbine is a function of fish species, size, and age as well as their physiological state and their physical location at entry to the runner environment, all convolved with the design of the turbine and how it is operated (Bishai 1961; Beyer et al. 1976; Cada 2001). These various biological and physical factors integrate to a unique physiological condition for each fish, the physical condition for any free or dissolved gas within the body of the fish, and the time history of pressure exposure during turbine passage.

It is possible to sort swim bladder-bearing fish into categories with varying degrees of susceptibility to barotrauma caused by rapid decompression given equivalent pressure exposures and physiological condition prior to exposure. One grouping of major importance is physostomous versus physoclistous. Physostomous fish have a connection (the pneumatic duct) between their swim bladder and esophagus (Figure 1.2) that permits expulsion of swim bladder air during decompression (Figure 1.2; Alexander 1966; Fänge 1966). Physoclistous fish (Figure 1.3) do not have such a duct connecting their swim bladder to the outside (Figure 1.3) and therefore do not have a way to rapidly modify the contents of their swim bladder when the external pressure changes (Jones 1951). These fish most likely also modify swim bladder air volume for other reasons. As a consequence, physoclistous fish are known to be considerably more susceptible to barotrauma than are physostomous species (Cada et al. 1997; Coutant and Whitney 2000). Salmonids are physostomous.

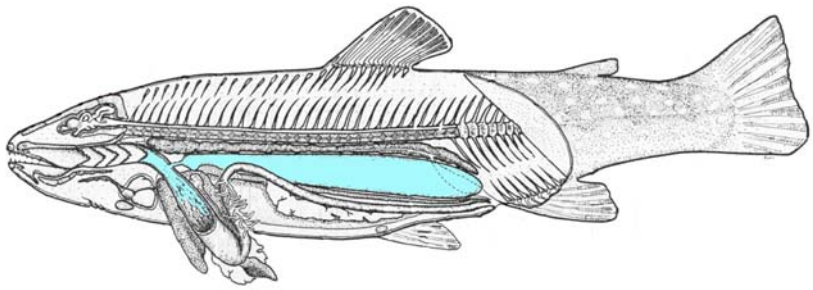


Figure 1.2. Diagram of a Physostomous Fish (trout) Showing (shaded in green) the Swim Bladder, Esophagus, and Pneumatic Duct Linking the Swim Bladder and Esophagus

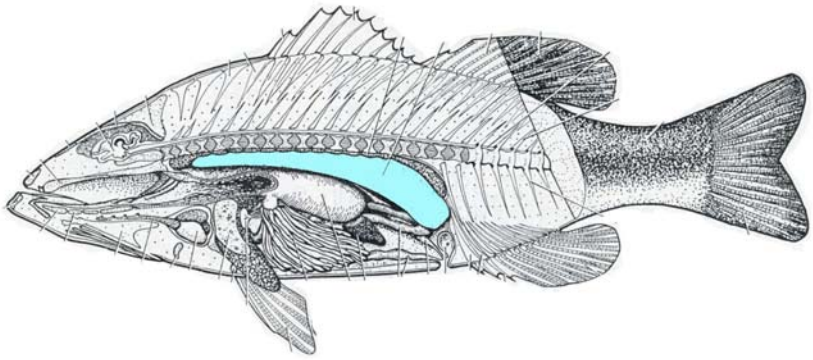


Figure 1.3. Diagram of a Physoclistous Fish (bass) showing (shaded in green) the Swim Bladder as an Organ Lacking any Structure to Facilitate Rapid Expulsion of Air

Neither physoclistous nor physostomous fish can volitionally alter the state of free and dissolved air in other organs and their bodily fluids other than through physical processes that control the movement of gas molecules across biological membranes and changes in state. Physostomous fish, unlike physoclistous fish, can burp air from their swim bladder, but this is not a transfer between organs and body fluids. The only stable endpoint of all gas exchange processes for body fluids and tissues is

equilibrium in dissolved gas tensions between the water surrounding the fish and the fluids within the fish.

In general, for the pressure changes fish experience during hydroturbine passage, the compression of air-filled structures and bubbles does not cause barotrauma; it is expansion of existing bubbles and formation of new bubbles that causes injury. Essentially all decompressive barotrauma is caused by changes in the state (in solution or free) and volume expansion of air-containing structures (e.g., swim bladder) or bubbles located elsewhere within the body of a fish in response to changes in pressure. The most important driving physical principals are 1) the relationship between the volume of a bubble or structure (swim bladder) and the change in pressure acting on the air-filled bubble or structure (Boyles' Law), and 2) the relationship between the solubility of gas in blood and other body fluids and the pressure acting on the fish (Henry's Law). Both the magnitude and direction of change in pressure are important.

There is an increase in the volume of any air-filled enclosure within the body of the fish when external pressure decreases. The change in volume is proportional to the magnitude of the change in pressure (Boyles' Law: $P_1V_1 = P_2V_2$ where P = pressure and V = volume). The rate of change in volume is essentially the same as the rate of change in pressure. Simultaneously, an increase in blood volume is caused by formation of bubbles from gas released from solution in the blood (Henry's Law: $P = k_gC$ or for a particular gas, $P_1C_2 = P_2C_1$; where P = pressure, C = concentration, and k_g = gas-specific solubility coefficient). The increased blood volume increases blood pressure in the arteries and veins and can disrupt the function of internal organs and biological processes essential for the survival of the fish. These physical phenomena in the body of a fish are the causes of commonly observed decompressive barotrauma injuries, which include, but are not limited to, rupture of blood vessels, bruising, severe physical damage to organs, swim bladder rupture, and occlusion of the circulatory system. Occlusion can include stoppage of the heart by bubbles lodging in the heart's chambers or partial or total shutdown of portions of the fish's circulatory and respiratory systems (Cramer and Oligher 1964; Tsvetkov et al. 1972; Beyer et al. 1976; Rummer and Bennett 2005). Injuries to other organs, including the gut where gas bubbles may normally exist, also occur.

For physoclistous fish, it has long been known that the depth of acclimation prior to pressure change exposure is a critical factor influencing the magnitude of injury and mortality rates (Beyer et al. 1976; Cada 1990; Rummer and Bennett 2005; Abernethy et al. 2001, 2002, and 2003). The reason for this is that acclimation to deeper depths requires physoclistous fish to transfer more molecules of air from their blood into their swim bladders to achieve swim bladder internal pressures and resulting volumes sufficient to reach displacements required for neutral buoyancy. When the mass of water displaced by a fish equals its mass, the fish becomes neutrally buoyant. 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; Lefrançois et al. 2001).

The susceptibility of physoclistous fish to barotrauma during hydroturbine passage, given acclimation to depth, has been found to be proportional to the ratio of absolute pressure at their acclimation depth to the lowest absolute pressure experienced during turbine passage (Cada et al. 1997; Abernethy et al. 2001, 2002, and 2003). For example, consider two fish, one acclimated to two atmospheres absolute (~30 psia), which would be the sum of hydrostatic and atmospheric pressure on a fish at a depth of approximately 10 m, and a second fish acclimated to near surface depth, which is equivalent to the absolute pressure of

approximately one atmosphere (~15 psia) or, for purposes of this discussion, atmospheric pressure only, not considering a small incremental addition resulting from very small submergence. Exposure to a turbine passage pressure nadir of 15 psia would have different barotrauma risk for these two fish even if all other physiological factors were exactly the same for both fish. For the fish acclimated to a 10-meter (m) depth, gas bodies within the fish would double in volume (ratio of acclimation to exposure pressure of 30/15 = 2) and gas in solution would be released into the blood in proportion to changes in solubility, while for the fish acclimated to near-surface conditions (ratio of 15/15 = 1), gas bodies would not change in volume nor would dissolved gas experience a change in state. These factors alone make it clear why fish acclimated to a deeper depth are more susceptible to decompressive barotrauma injury than fish acclimated to lesser depths.

Studies of the effects of pressure change during hydro-turbine passage on physostomous fish, particularly juvenile salmonids, have not included depth acclimation as a factor (Sutherland 1972; Abernethy et al. 2001; Abernethy 2002, Abernethy et al. 2003). The reasons for this omission are not clear but have occurred in spite of a very large body of information that clearly shows that Federal Columbia River Power System juvenile salmonids are distributed in the upper portion of the water column down to depths of 40 ft and greater.

In this study, we have included acclimation depth as a treatment factor in our study of barotrauma based on the assumption that some or all of the juvenile salmonids observed to be present at a depth are buoyancy-acclimated to that depth. To our knowledge, there is no direct evidence to either support or refute this assumption for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) migrating down the Columbia River or in any other location. In our study, we hypothesized that the risk of barotrauma injury and mortality to juvenile Chinook salmon during simulated turbine passage is higher for fish acclimated to higher absolute pressures. The data to be presented clearly show that this is a critical assumption in assessment of barotrauma for downstream-migrating juvenile salmonids.

To estimate dam passage survival, juvenile salmonids migrating down the Columbia and Snake rivers are often implanted with radio-transmitters or other telemetry devices. The addition of an internally implanted transmitter to a juvenile salmonid may increase the likelihood of barotrauma injury or other damage during turbine passage. A transmitter adds mass to the fish which the fish must compensate for by increasing its displacement to achieve neutral buoyancy. Increases in buoyancy are achieved by the fish gulping air into its swim bladder, which causes changes in the volume of the fish and thereby increases the buoyant force acting on the fish (Perry et al. 2001; Anglea et al. 2003; Brown et al. 2005). The presence of the transmitter and increased swim bladder volume for depth-acclimated fish may create conditions that will increase, relative to untagged fish, the probability for barotrauma injury and other damage to tagged fish when they are exposed to low pressures during turbine passage. A transmitter also occupies volume in the body cavity of a fish. This may increase the risk of injury to internal organs when fish are exposed to low pressures during turbine passage. We hypothesized that the risk of injury and mortality to fish bearing internal telemetry transmitters exposed to simulated turbine passage pressure is higher than for untagged fish.

Telemetry transmitters are implanted using two different methods. One method is surgical implantation where an incision is made into the body wall of a fish, the telemetry device is inserted into the fish's peritoneum and the incision is closed using sutures. The second method is gastric implantation,

which does not require surgery. In gastric implantation, a telemetry device is placed in a fish's stomach through its mouth. We hypothesized that the risk of barotrauma injury and mortality to fish bearing radio transmitters during exposure to simulated turbine passage pressure would not be different for the two types of transmitter implantation.

1.2 Objectives

This study is an element of the Turbine Survival Program. The overall goal of the Turbine Survival Program is to provide guidance to USACE efforts during rehabilitation, replacement, and day-to-day operation of hydro turbines within the Federal Columbia River Power System to optimize the survival of downstream migrating juvenile salmonids passing through the turbines. This study was designed to initiate investigation of the effects of rapid decompression on river-run juvenile salmonids caused by exposure to low pressures during turbine passage. Specifically it addresses the combined effects of depth acclimation, presence of a telemetry device, method of telemetry device implantation, and juvenile fish age (and therefore size) on the risk of barotrauma upon exposure to rapid decompression for fish passing very near the suction side of runner turbine blades. The intent of the study was to test the significance of the various experimental factors for risk of barotrauma, not to provide point estimates of that risk.

1 Evaluate the response of depth-acclimated run-of-the-river yearling Chinook salmon tagged using gastric and surgical implantation methods with a radio telemetry micro-transmitter to simulated turbine passage pressure cycling.

2 Evaluate the response of depth-acclimated run-of-the river subyearling Chinook salmon tagged using gastric and surgical implantation methods with a radio telemetry micro-transmitter to simulated turbine passage pressure cycling.

1.3 Overview of this Report

Chapter 2 provides methods, Chapter 3 is results, Chapter 4 is discussion, Chapter 5 is conclusions, and Chapter 6 is references.

2.0 Methods

This study was conducted with run-of-the-river juvenile Chinook salmon between April 26 and August 8, 2005. Both yearling and subyearling fish were obtained from the Juvenile Passage Facility at McNary Dam (Figure 2.1) with the aid of Washington State Department of Fish and Wildlife personnel.

Figure 2.1. U.S. Fish and Wildlife Juvenile Passage Facility at McNary Dam

2.1 Test Fish

Simulated turbine passage (STP) pressure exposure testing was conducted between April 25 and August 7, 2005, with 516 subyearling Chinook salmon and 512 yearling Chinook salmon (Table 2.1). Yearling Chinook salmon were exposed to simulated turbine passage treatments between April 25 and June 20, 2005. The yearling Chinook salmon used in the study had a mean length of 152.6 millimeter [mm] (range 126-203 mm; SD=12.4; SE = 0.55; Figure 2.2) and a mean weight of 34.7 g (range 14-79 grams [g]; standard deviation [SD]=9.1; standard error [SE]=0.4) (Figure 2.3). Subyearling Chinook salmon were exposed to simulated turbine passage treatments between June 21 and August 7. They had a mean weight of 15.1 g (range 10-29 g; SD= 3.4; SE=.15) and a mean length of 112.4 mm (range 99-139 mm; SD=7.6; SE=0.33; Figure 2.3). During the study, test fish were exposed to simulated turbine passage pressure exposure treatments that included three different transmitter factors. Transmitter treatment factors were 1) gastrically implanted radio transmitter, 2) surgically implanted radio transmitter, and 3) fish not implanted with a radio transmitter.

Table 2.1. Number of Fish Used in the Study by Fish Age, Transmitter (tag) Implantation Method, and Exposure to Simulated Turbine Passage Pressure

Tag Treatment	Subyearling Chinook		Yearling Chinook		Total
	STP Exposure	No STP Exposure	STP Exposure	No STP Exposure	
Gastric Implantation	83	83	80	101	347
Surgical Implantation	85	88	78	83	334
No Tag	91	96	84	86	357
Total	259	267	242	270	1038

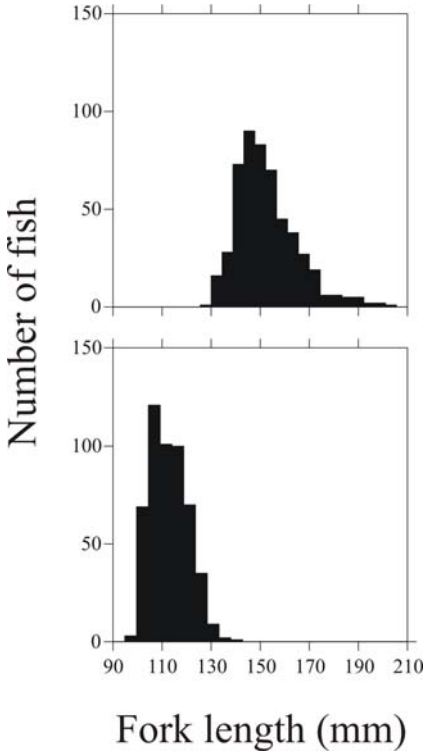


Figure 2.2. Length Distribution of River-Run Yearling (upper panel) and Subyearling (lower panel) Chinook Salmon

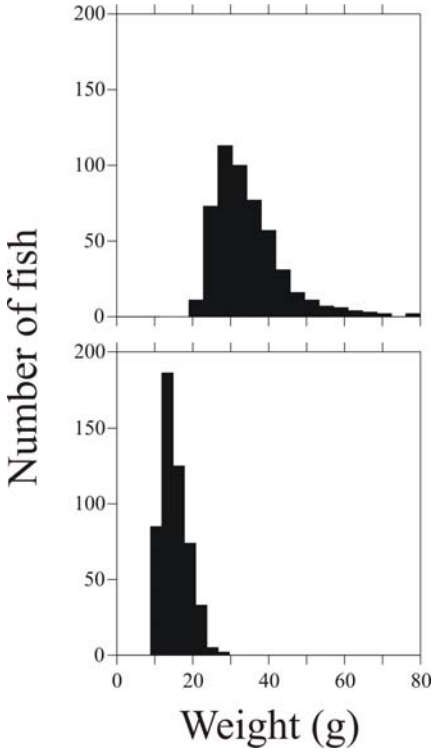


Figure 2.3. Weight Distribution of River-Run Yearling (upper panel) and Subyearling (lower panel) Chinook Salmon

Transmitters were implanted by U.S. Geological Survey (USGS) staff at McNary Dam using the methods of Adams et al. (1998). Following implantation, Battelle staff freeze branded each fish with a unique external mark to provide a means to visually identify each individual fish during observation through acclimation, exposure, and post-exposure holding periods.

2.2 Transmitters

The 342 yearling Chinook salmon were implanted with model NTC-3-1 transmitters (Lotek Wireless, Newmarket, Ontario, Canada), which had a mean weight of 1.02 g in air and 0.51 g in water and a volume of 0.55 milliliters (ml). These transmitters added 1.3% to 4.7% (a mean of 3.1%; SD = 0.7; SE = 0.03) to the body weight (mass) of the test yearling Chinook salmon (Figure 2.4).

The 339 subyearling Chinook salmon were implanted with model NTC-M-2 transmitters, which had a mean weight in air of 0.42 g in air and 0.23 g in water and a volume of 0.19 ml. These transmitters added 1.4% to 4.2% (a mean of 2.9%; SD=0.7; SE=0.03) to the body weight (mass) of test subyearling Chinook salmon (Figure 2.4).

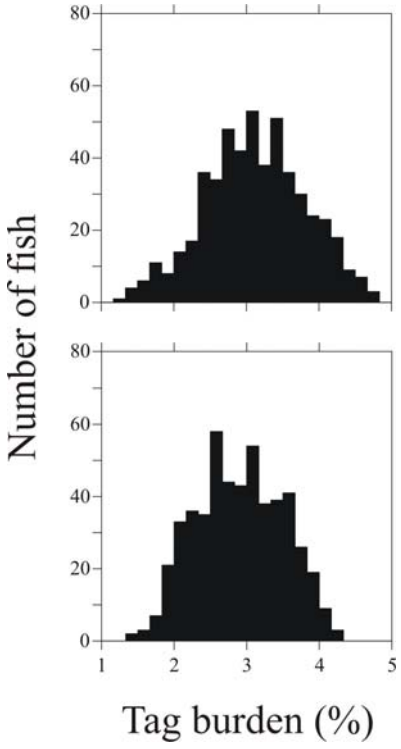


Figure 2.4. Distribution of Ratio of Radio Transmitter Weight to River-Run Yearling (upper panel) and Subyearling (lower panel) Chinook Weight

2.3 Pressure Chamber Set Up

The STP tests were conducted in a pair of pressure chambers (Figure 2.5) designed and built by Reimers Engineering of Springfield, Virginia. The system consisted of two 27.5-cm-diameter acrylic tubes, 55 cm long with a volume of 34 liters each. Hydraulic cylinders connected to pneumatic cylinders (Figure 2.6) were moved under the control of a computer (programmed using Labtech Laboratory Technologies Corporation, Middleboro, Massachusetts) to either pressurize or depressurize the chambers (i.e., hyperbaric and hypobaric conditions respectively). The system could reduce the pressure in the chambers from ~60 psia (414 kPa) to near the vapor pressure of water ~1 psia (~7 kPa) in 0.1 sec. This equaled a rate of change in pressure during the maximum pressure reduction phase of the STP of approximately 590 psi/sec (4,070 kPa/s).

2.4 Water Supply

Ambient river water supplied via the juvenile passage facility at McNary Dam was delivered to our pressure testing facility at a pressure of ~34 psi. The temperature of the river water increased from 11.4°C to 16.9°C during the yearling test and from 17.8°C to 22 °C during the subyearling test. The level of total dissolved gas (TDG) in the water supplied to the pressure testing facility was within the range of 101% to 102% (Table 2.2), although total dissolved gas levels were higher (108% to 111%) in the forebay of the dam. Forebay water was degassed as it passed through the juvenile fish facility, which reduced its TDG to the levels we observed at the input to our test facilities. We did not attempt to modify the temperature or the TDG condition of the water prior to use. Upon receipt from the juvenile fish facility,

water was filtered using a 0.16 cm² mesh screen to remove larger debris then pumped to the pressure chambers using a centrifugal pump. The centrifugal pump was placed in line with the water supply to ensure little or no water pressure variability during the testing period. A valve on the outlet side of the centrifugal pump was used to maintain the supply of an appropriate volume of pressurized water to the pressure chambers' inlet piping system. Following the pump, the water plumbing was split to provide water to both pressure chambers through manual and electrically controlled inlet valves (Figure 2.7).

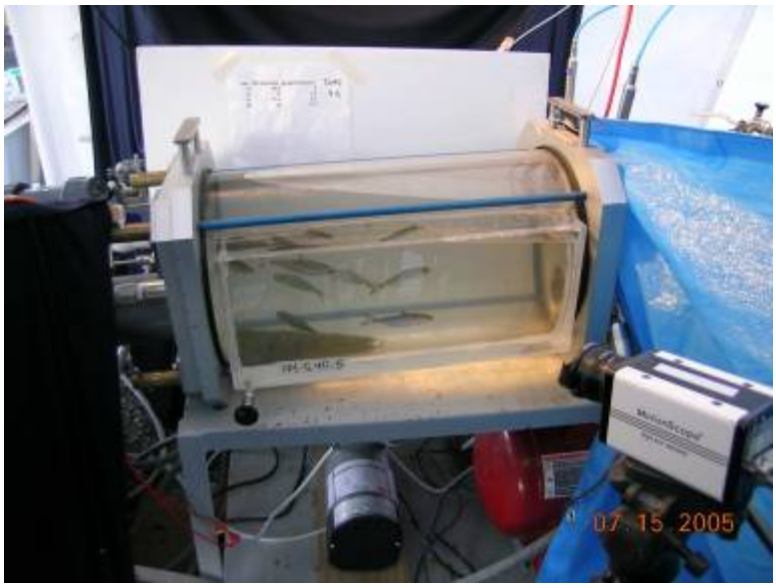


Figure 2.5. One of Two Pressure Chambers Showing Fish during Acclimation with an Air Pocket Present in the Chamber

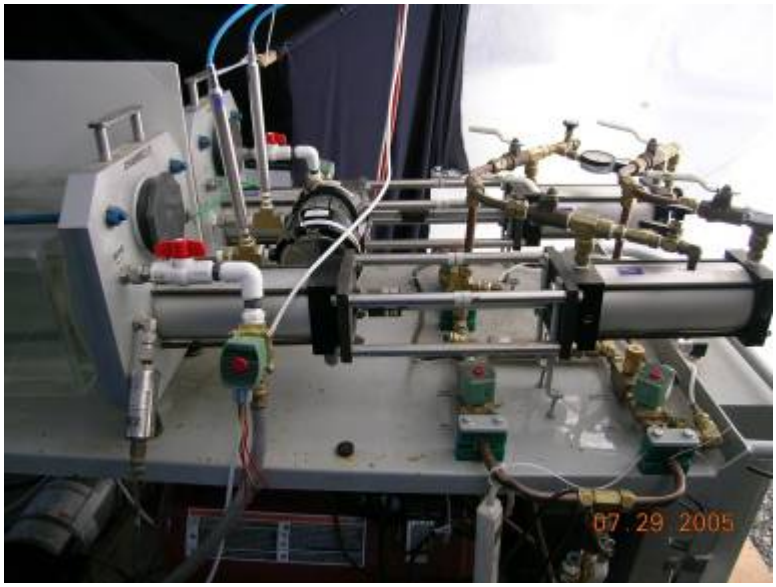


Figure 2.6. Hydraulic Cylinders Connected to Pneumatic Cylinders

Table 2.2. The Mean Temperature (°C) and Total Dissolved Gas (% saturation) (±SD) at Receipt into the Pressure Testing Facility

Location	Age	Total Dissolved Gas TDG	Total Dissolved Gas Range	Water temperature Temperature	Water temperature Range
Testing Station					
	Yearling	101.5 ± 2.0	99.5 to 108.5	14.7 ± 1.2	11.4 to 16.9
	Subyearling	101.2 ± 0.8	100.1 to 103.5	20.1 ± 1.3	17.8 to 22
Forebay					
	Yearling	108.3 ± 4	100.9 to 117.7	10.1 ± 3.7	3.6 to 15.9
	Subyearling	110.6 ± 1.3	107.5 to 114	19.2 ± 1.4	16.7 to 21.3

Figure 2.7. Water Inlet Valves Controlling Water Entering the Hyperbaric Chamber

2.5 Loading Fish

The next step in the test procedure was to load the test fish into the chambers. Typically seven fish with radio transmitters were loaded into one chamber and seven without transmitters were loaded into the other. The number of fish per chamber was limited so that all fish could be visually observed during acclimation and testing. In addition, it was necessary to limit the number of fish within a chamber to stay within the ability of the chamber to accommodate the compliance added by the air in the fish's bodies and still accurately reproduce the desired STP pressure sequence. Test fish were netted from holding tanks (~18 liter volume) and placed into a bucket containing ~5 liters of water. The water containing the fish was then poured from the bucket into a chamber through the chamber's 10.2-cm PVC drain valve (Figure 2.8) which was turned upward 180º from the position shown in Figure 2.8. The process was then repeated for the remaining chamber. After the test fish were placed inside the chambers, the chambers' 10.2-centimeter (cm) PVC drain valves were closed and reoriented to their downward discharge position, and the centrifugal pump was energized. Water was then allowed to flow into the chamber by opening

the inlet and outlet valves. Manipulation of these valves permitted control of the water flow rate and pressure in the chambers. The chambers were allowed to fill, while retaining an air pocket, and their pressure was set to the desired acclimation pressure. Fine tuning of water flow through the chambers and of pressure in the chambers was accomplished by making small adjustments to the gate valves (Figure 2.9) positioned outside the outlet valves on both chambers.

Figure 2.8. PVC Drain Valves (10.2 cm; orange handles illustrated by white arrow) Mounted on the End of the Hyperbaric Chambers

Figure 2.9. Adjustable Gate Valves

2.6 Acclimation Prior to Pressure Exposure

The test fish were acclimated at pressures equivalent to the absolute pressures that would exist at four different depths in fresh water given standard atmospheric pressure and fresh water density at a temperature of 39°C: Zero feet (or standard atmospheric pressure, 14.7 psia or 101 kPa); 10 feet (19 psia or 131 kPa); 20 feet (23.4 psia or 161 kPa); and 40 feet (32 psia or 221 kPa). As a chamber was filled, inflow was controlled to permit a small air pocket (Figure 2.5) to form within each pressurized chamber. The air pocket was accessible to test fish through the acclimation period so that they could gulp air to fill their swim bladders and thereby modify their displacement and buoyancy. Test fish were held in the chambers for 16 to 24 h to allow ample time to acclimate for buoyancy and equilibration of gas tensions in bodily fluids and tissues. Acclimation pressure within the chambers was continuously monitored using pressure sensors located in the chambers. The analog outputs from the pressure sensors were digitally sampled and recorded using a digital signal processing card installed in a computer. Chamber pressures were recorded at ~100-sec intervals through the acclimation period. In addition to the manufacturer's pressure sensors located in the pressure chambers, an additional pressure sensor (model PT2X; INW, Kirkland, Washington) was installed within each chamber. The outputs of these additional pressure sensors were recorded to electronic files using software running on a laptop computer. These pressure sensors were completely independent of the pressure sensors installed in the chambers by the chamber manufacturer. The laptop ran a software program, Aqua-4 Plus (INW, Kirkland, Washington), to acquire and process the pressure sensors' outputs, which resulted in a second series of pressure measurements obtained at regular intervals for each chamber.

2.7 Simulated Turbine Passage Exposure

At the conclusion of a depth acclimation period, STP exposure was initiated using a computer program that controlled the chamber's hydraulic and pneumatic cylinders. This program controlled the cylinders to increase pressure to ~58 psi (400 kPa) over 30 to 60 s to simulate fish passing through a hydroelectric turbine intake and approaching the turbine runner (Figure 2.10). The program then controlled the chambers' pistons to subject the fish to a sudden decrease in pressure to ~ 1 to 3 psia (2 to 10 kPa), the nadir in pressure that would be experienced by passage of the test fish through a McNary Dam turbine runner near the suction side of a runner blade. Finally, the program controlled the chamber pressure to simulate movement of fish out of the turbine draft tube, into the tailrace, and up to the water surface where pressure was returned to atmospheric pressure (~14.7 psia, 101 kPa). The time required for a STP sequence was 60 to 90 s depending on the time required for pressure changes from acclimation pressure to the maximum pressure for simulated turbine intake passage. These STP scenarios were the same as those computed by Montgomery Watson (1995) and used by Abernethy et al. (2001) to simulate passage of fish near the suction side of McNary Dam turbine runner blades. The exposure scenario nadir represents the worst case conditions for the low pressure exposure juvenile fish would experience during passage through an operating McNary Kaplan turbine.

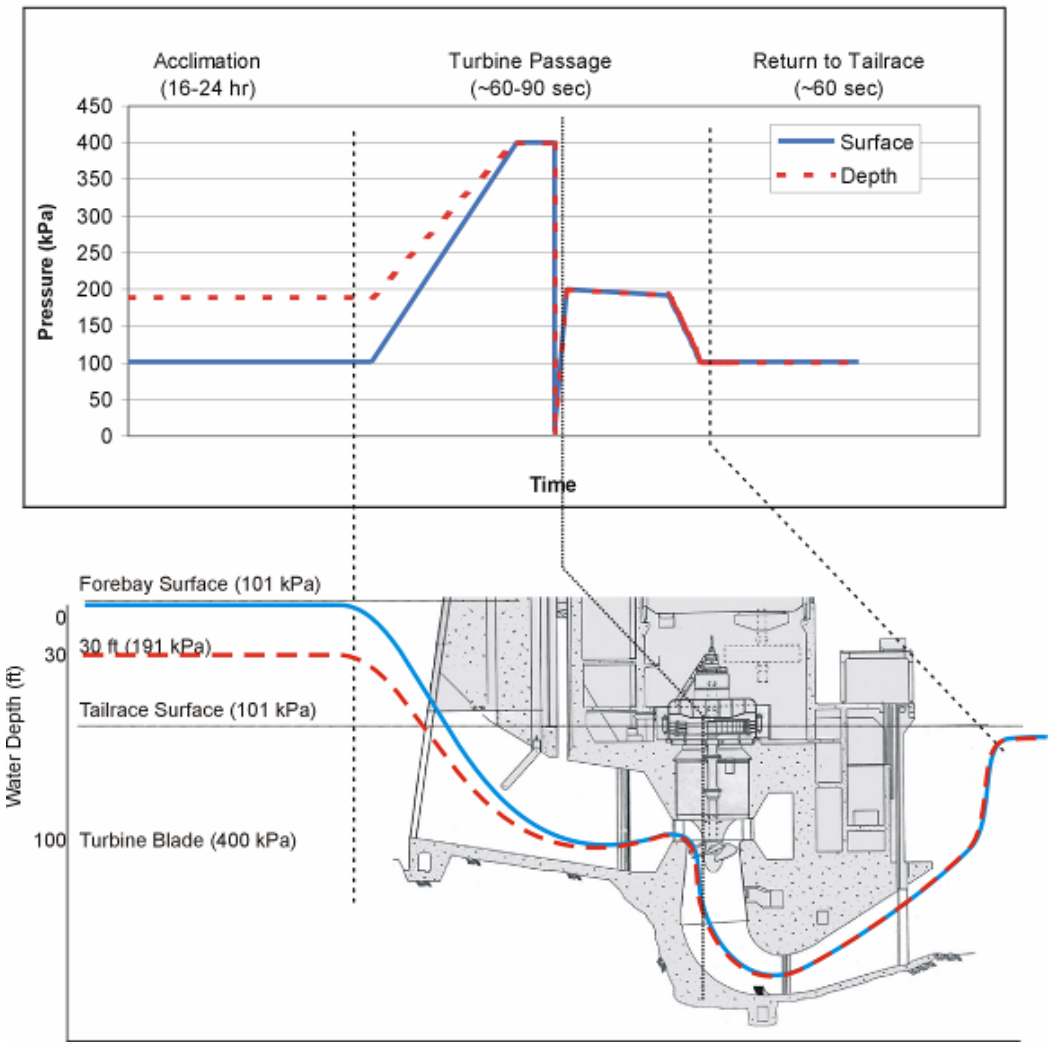


Figure 2.10. Two Examples of Trajectories through a Kaplan Turbine Used to Compute STPs, a Surface Acclimation Trajectory (blue) and a 30-ft Depth Acclimation Trajectory (red dashed). In these trajectories, fish are assumed be passively transported through the turbine runner by water flow in approximately 60 to 90 seconds, depending upon depth of entry to the turbine intake (acclimation depth). Following passage through the turbine runner, turbine-passed fish exit through the turbine draft tube, enter the tailrace, and go up to the water surface, which takes approximately 60 additional seconds. The upper graph shows the pressure changes the fish undergo during turbine passage. The rapid decrease in pressure and the nadir in the STP occurs during passage through the turbine runner (blade assembly).

2.8 Fish Removal

Upon completion of STP exposure, the chambers' needle valves were slowly opened to release the pressure in the chambers then the 10.2-cm drain valves were opened to ensure atmospheric pressure within the chambers. Once atmospheric pressure in the chambers was achieved, the chambers' inlet and outlet valves were opened, water was allowed to flow freely through the chambers, and the test fish were removed from the chambers.

2.9 Water Quality Monitoring

Directly after the STP sequence was completed, the water temperature and total dissolved gas (%TDG) of the water from the pressure chambers was measured using a Sweeney Aquametrics-Model BS1-B saturometer (New Haven, CT).

The test fish were then released from the pressure chambers through the 10.2-cm PVC drain valves and into a net submerged in a large trough. The trough was positioned to catch the water discharged from the pressure chambers. The test fish were netted and placed in clear recovery tanks containing filtered river water at ambient TDG and temperature conditions for water supplied to the test facility for a 48-hour post-exposure observation period (Figure 2.11).

Figure 2.11. Clear Recovery Tanks (18 liter) Used for the 48-Hour Post-Exposure Observation Period

2.10 Visual Observations

2.10.1 Determination of Buoyancy

The buoyancy of the fish prior to STP exposure may influence the amount of damage caused by rapid decompression during simulated turbine passage. To determine the buoyancy of the fish prior to STP exposure, video images were analyzed. The behavior of each individually identifiable fish was observed to determine if it was negatively, positively, or neutrally buoyant before and after STP exposure. Negatively buoyant fish tend to swim "head-up/tail-down" to keep off the bottom of the chamber and they also had elevated tail beat rates (Harvey 1963; Figure 2.12). Neutrally buoyant fish were able to maintain a horizontal position within the chamber or holding tank with minimal fin movement. Symptoms of positive buoyancy were exhibited by fish continuously struggling to move down in the water column (head down/tail up) (Harvey 1963).

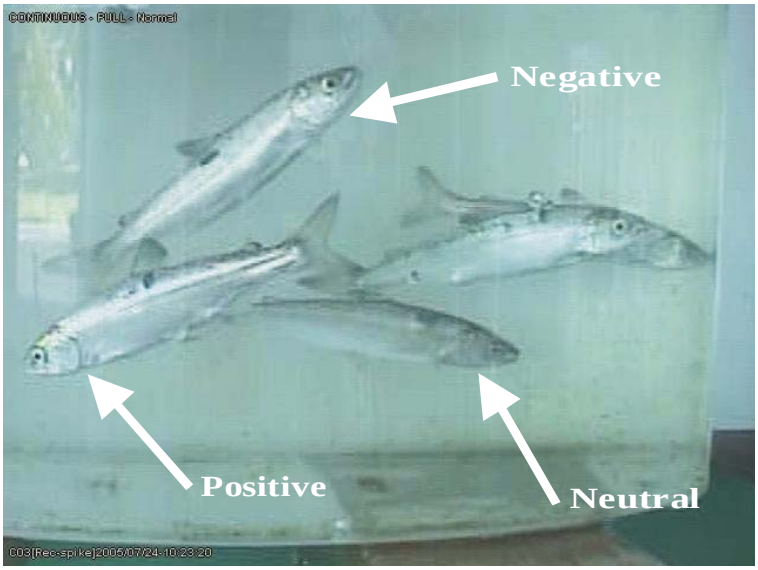


Figure 2.12. An Image of Untagged Fish Exhibiting Different States of Buoyancy Shortly Following Placement into a Pressure Chamber

2.10.2 Image Recording during Simulated Turbine Passage

Video recordings of each lot of test fish were made at several times throughout the testing sequence from first placement into the test chamber until just prior to necropsy following the 48-hour post-STP exposure holding period. These records were processed to observe the behavior and condition of fish throughout each of the STP testing phases. The times and durations of video recording during each phase of treatment to evaluate the condition of test fish are shown in Table 2.3. Careful control during replay of the video records allowed each individual fish to be observed. In particular, high-speed digital recording of test fish during the STP and playback at slower speeds permitted the condition and response of each test fish to pressure changes during STP to be observed in considerable detail.

Table 2.3. Speed and Time Length of Video Recordings during each Phase of Pressure Exposure Sequence

Study phase	Imagery type		Recording time
	High speed	Standard speed	
Acclimation			
Initial acclimation		X	1 h
Pre STP		X	20 min
During STP	X	X	4 s
Recovery			
Post STP		X	2 h
Pre- necropsy		X	10 min

Fish behavior was digitally recorded with either high-speed or standard Hi 8 video cameras. The type of video system used depended on the stage of the experiment (Table 2.3). During all phases of the

experiment, observations were made at standard video frame rates (i.e., 30 frames/s using a digital video recording system, Open Eye, Spokane, Washington). However, during the portion of STP for turbine runner passage, a high-frame-rate camera system was also used (RedLake, Tucson, AZ). High-frame-rate video was recorded at 250 frames/s. At this video recording frame rate, detailed observations of the response of each individual fish to the very short (approximately 100 millisecond) portion of the pressure exposure that simulated passage through a turbine runner could be observed. This portion of the pressure exposure contains the most rapid changes in pressure and the nadir in pressure for STP exposure.

Standard video rate recording of test fish was done for the first hour during the 16- to 24-hr acclimation period following a period of study where it was determined that this length of observation was sufficient to observe the behavior of test fish when they were gulping air and adjusting their buoyancy. A second period of standard video rate observation was conducted during a 20-minute period immediately prior to STP exposure at the end of the acclimation period. Video recordings were used to observe test fish behavior during acclimation (buoyancy adjustment) to the various depth-equivalent pressure treatment factors. Test fish were observed to swim up to the air pocket in the air chamber and gulp air to attain neutral buoyancy during acclimation. Other observations of fish behavior included the general activity level of each fish, which ranged from constant swimming to lying on the bottom of the chamber throughout the acclimation period.

The second standard video rate observation period, at the end of the acclimation period and immediately prior to STP exposure, was used to evaluate the buoyancy condition of each test fish. Observations of the state of buoyancy of each test fish were obtained for later analysis to investigate any relationship between buoyancy state prior to STP exposure and the outcome of STP exposure.

During STP, high-frame-rate video observations of test fish were obtained. These recordings were analyzed by playing them back frame by frame to observe the response of each test fish to simulated turbine runner passage. Test fish began to respond to changes in pressure almost immediately upon initiation of a STP sequence. Initial behavioral responses were swimming upward in the chamber as pressure was increased in the early portion of the STP sequences. Observed test fish responses to rapid decompression during simulation of runner passage included coughing, violent shaking of the head, convulsions, very rapid startle response like swimming upward in the chamber, tag expulsion from the mouth in the case of gastric implants or through the surgical incision for surgically implanted transmitters, and air expulsion. Air was observed to be expelled from the mouth, under gill covers, out of the vent, through the surgical incision, or some combination of all four.

Test fish were also observed during the 16- to 24-hr post-exposure holding period using standard frame rate video recording for a two-hour period immediately following placement into holding aquaria and, for those fish that survived over the holding period, for 10 minutes prior to necropsy. Holding period video records were processed to observe post-STP exposure effects.

Video records during the initiation of holding immediately following removal from pressure chambers were analyzed to determine if behaviors such as coughing, violent shaking of the head, convulsions, or loss of equilibrium and buoyancy were present. In addition, video records of all gastrically implanted test fish were examined to determine if any fish expelled their transmitter during this period following STP exposure. The second critical time period for observation of fish condition was

immediately pre-necropsy. At the end of the holding period, video records were obtained to document test fish buoyancy and equilibrium condition.

2.11 Necropsy

Any fish that died during STP exposure or during post-exposure holding were immediately necropsied to determine the cause of death (Figure 2.13). At the end of the 48-hour holding period, any test fish still alive were euthanized with tricaine methane sulfonate (MS-222) and necropsied to determine condition and detect any significant sublethal injuries that may have occurred.

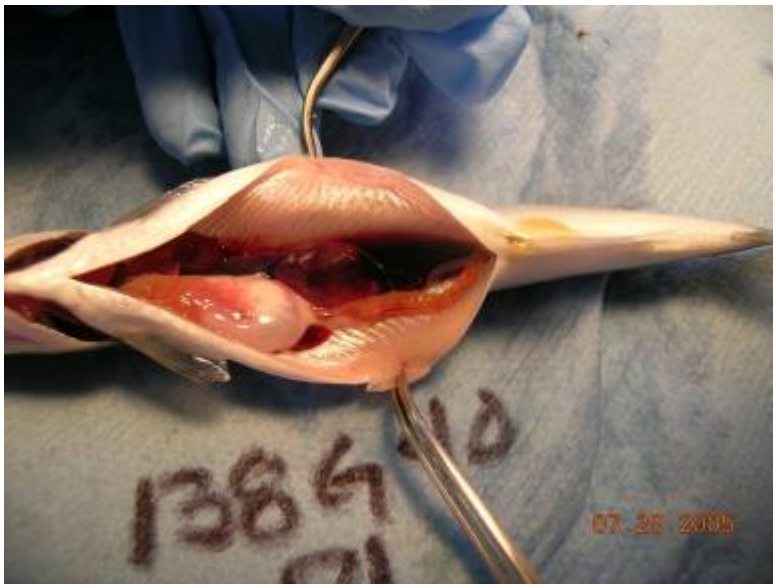


Figure 2.13. Necropsy Being Performed on a Test Fish that Died during Pressure Exposure during the STP Sequence

Necropsy procedures included external examination for bubbles in the fins, eyes, gills, and mouth and hemorrhaging in the fins, eyes, and gill filaments. External observations of the spleen in relation to the surgical incision were also made. Internal examination included examining fish for swim bladder rupture and the location and extent of rupture, the location and severity of hemorrhages, and damage to the spleen or other organs. Observations also were made for the occurrence and severity of bacterial kidney disease. Additional observations were made to identify injuries or tissue response to implantation or the presence of radio transmitters. These observations included inspection for the routing of the transmitter antenna and any damage to the test fish's swim bladder or other organs that may have occurred during surgical transmitter implantation.

2.12 Histology

Sixty-nine fish died during or shortly after the STP; these fish were examined using histological techniques to determine damage to fish from STP exposure. The condition of the swim bladder and visceral organs of these 69 test fish was systematically investigated by taking a number of cross-sections from the fish for histological examination. Four transverse sections were made for the first 18 test fish histologically examined. These cross-sections were taken in the approximate locations shown in

Figure 2.14. The figure shows only approximate locations and there was not necessarily a larger space between sections 3 and 4, as shown in Figure 2.14.

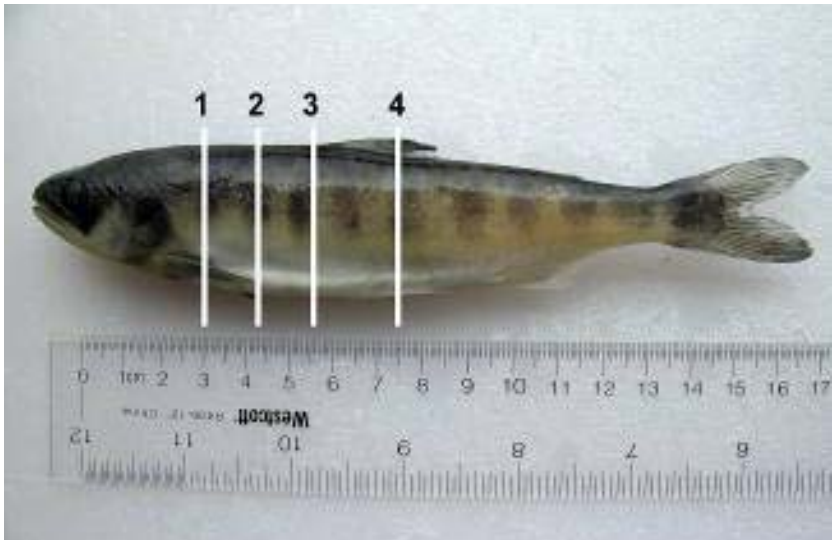


Figure 2.14. Approximate Location of Traverse Sections Taken from the First 18 Test Fish that Were Histologically Examined

Following histological examination of the first 18 fish, it was determined that three cross-sections in the body region between cross-sections 1 and 4 shown in Figure 2.14 would be sufficient for histological examination of the remaining 51 fish. In addition to these three cross-sections, one saggital section of the head was included to examine for lesions or tissue damage to the heart, ventral aorta, and gills. The saggital section ran from the tip of the snout to the posterior margin of the operculum along the midline of the fish. The saggital sections bisected the head of the test fish lengthwise into mirror images, one containing the left eye and one containing the right eye.

A single, very thin section was taken from each histological block made from each cross-section and stained with hematoxylin and eosin dyes. The anterior-most cross-section was located at the anterior aspect of the visceral cavity, thus the series of sections included skeletal muscle, spinal cord, caudal artery and vein, kidney, swim bladder, liver, reproductive tracts, elements of the gastrointestinal tract, and spleen. Other minor organs were also present in some but not all sections. All organs present were examined for anomalies and lesions. Histological examination is a considerably more sensitive method for detecting small and microscopic lesions and other tissue damage than necropsy examination, even with the aid of a stereoscope during necropsy.

2.13 Multi-Factor Study Design and Statistical Analysis

The effects of simulated turbine passage were studied on 1,010 juvenile Chinook salmon (512 subyearlings and 498 yearlings). The factors examined in this study were 1) acclimation depth-equivalent absolute pressure, 2) the presence or absence of an internal radio telemetry transmitter, 3) transmitter implantation method, and 4) exposure to simulated turbine passage (Tables 2.4 and 2.5).

Table 2.4. Target Sample Sizes of Fish Exposed to Simulated Turbine Passage (three replicates of seven fish = 21 fish). Fish were exposed to one of three different radio transmitter tagging treatments and one of four acclimation pressures.

	Exposed to simulated turbine passage				
	pressure		tag treatment		
depth of water (feet)	psia	kPa	non-tagged	gastric	surgical
0	14.7	101	21	21	21
10	19.0	131	21	21	21
20	23.4	161	21	21	21
40	34.0	234	21	21	21

Table 2.5. Target Sample Sizes of Fish not Exposed to Simulated Turbine Passage (three replicates of seven fish = 21 fish). Fish were exposed to one of three different radio transmitter tagging treatments and one of four acclimation pressures.

	Not exposed to simulated turbine passage				
	pressure		tag treatment		
depth of water (feet)	psia	kPa	non-tagged	gastric	surgical
0	14.7	101	21	21	21
10	19.0	131	21	21	21
20	23.4	161	21	21	21
40	34.0	234	21	21	21

Separate studies were conducted for yearling and subyearling river-run Chinook salmon. Fish were exposed to one of three tagging treatments (surgical, gastric, and not implanted), acclimated at one of four acclimation pressures, and then either exposed or not exposed to STP in a balanced and replicated experimental design. Each replicate consisted of 24 treatment combinations with seven fish each: four acclimation pressures by three tagging methods by two levels of STP (i.e., exposure or not) resulting in 24 different combinations (see Tables 2.4 and 2.5). At least three replicates were conducted of each of these combinations. Data were only used in the following analysis if pressures reached 3.0 psia or lower during the nadir of simulated turbine passage.

Daily tests were conducted consisting of 14 fish, all depth acclimated and tagged (or not tagged) in the same manner, with seven exposed to the STP sequence in one chamber, and the remaining seven placed in the other chamber and not exposed to STP. These latter fish (control sample) were used to account for the effects of handling, tagging, and depth acclimation as separate from the effects of STP.

Each chamber of fish comprised a binomial trial with the number of events (e.g., mortality or specified injury) per trial modeled using logistic regression (Hosmer and Lemeshow 2000). The design matrix for this model included acclimation depth and the three levels of radio transmitter implantation method (surgical, gastric, or no tag) as a three-level nominal factor variable, with simulated turbine passage (STP) entered as a dichotomous factor variable. In some analyses, the two age classes of fish were combined into the same model as a dichotomous factor variable. Since the focus of this study is

primarily on the effects of tagging, age class, and STP; acclimation depth was entered into the model as a continuous predictor variable. This allowed the effects of acclimation depth to be more efficiently estimated incrementally using only a single degree of freedom to estimate its model coefficient. Wald chi-square tests for differences in event rates were based on the estimated coefficients from the fitted model. For factor variables, comparisons were made using the "reference cell coding" and odds-ratio methods described in Hosmer and Lemeshow (2000). Goodness-of-fit was assessed in each fitted model using the estimated scale parameter. All reported p-values are from Wald chi-square tests unless otherwise noted.

In separate analyses on the effect of state of buoyancy before STP and expulsion of air during STP, mortality and specified injuries were coded as binary on individual fish and a similar logistic regression model was used to assess differences in rates between factors.

2.14 Other Experiments

2.14.1 Healing of Swim Bladder Tears

Between September 9 and October 21 of 2005, an experiment was conducted at the PNNL wet lab to determine if damage to swim bladders would heal and to obtain initial observations of the time to heal. Fifty juvenile rainbow trout available at the PNNL hatchery were included in this experiment. A tear was made in the swim bladders of 40 of the juvenile rainbow trout while the remaining 10 served as controls. A surgical incision was made in the sides of the test fish and tears were made in their swim bladders using a pair of scissors. In each case, the tips of the closed scissors were inserted into the fish's swim bladder and then the scissor blades were opened to create a tear about ~ 5 to7 mm in length. The surgical incision in the side of the fish was then closed with two 5-0 vicryl sutures. The control fish were surgically incised then sutured in the same manner as the test fish but no hole was torn in the swim bladder.

Test fish were held in 16°C water for 28 days. Every 7 days, a group of 10 fish was necropsied to determine the status of their swim bladders. On day 28, along with the fourth group of 10 fish, the 10 control fish were necropsied. Results of the necropsies were used to determine if the damaged swim bladder would heal and the rate of healing.

During necropsies, the swim bladders of test and control fish were examined for holes and scar tissue, and the degree of inflation. The swim bladder was completely removed from some test fish for examination. This was done by cutting the swim bladder anterior to the pneumatic duct and carefully removing it from the body cavity.

2.14.2 External Mass Experiment

An additional experiment was conducted to observe the response to STP of river-run subyearling Chinook salmon bearing an externally attached dummy transmitter. This research was conducted with 61 subyearling river-run juvenile Chinook salmon between July 28 and August 8, 2005. The test fish had a mean weight of 17.9 g (range 12.7-26.6 g; SD=3.4) and a mean length of 116.6 mm (range 104-130 mm; SD=6.5).

A dummy acoustic transmitter was externally attached to the test fish. Test fish were sedated with 80 mg/l MS-222, weighed, measured, and branded for identification and, in some cases, the upper or lower

caudal fin was clipped for identification. The dummy transmitter was attached to the ventral side of the fish just anterior to the dorsal fin using a 5-0 Vicryl suture.

The dummy transmitters were lead fishing sinkers trimmed to a weight of 0.35 g. The sinker was crimped onto the suture located on the fish's stomach anterior to the leading edge of the base of the dorsal fin.

After tagging, fish were allowed to recover in fresh water and were then loaded into the pressure chambers. Different lots of 7 test fish each were acclimated prior to STP exposure (using methods described earlier) to the absolute pressure present at 0 feet or the water surface (14.7 psia; 101 kPa), 10 feet (19 psia, 131 kPa), and 20 feet (23.4 psia, 161 kPa). After acclimating for 1.5 to 2 hrs, the fish were exposed to STP with a nadir between 1 and 3 psia (2 to 10 kPa). Post-STP holding and necropsy were conducted as described previously.

2.14.3 Short Acclimation Experiment

A small pilot study was also conducted to investigate whether the mortality rate of fish limited to 2 hours for depth acclimation would be significantly different from the mortality rate of fish allowed 24 hours to depth acclimate. This research was conducted with river-run subyearling Chinook salmon August 3-9, 2005. The 42 subyearling fish used for this test had a mean weight of 17 g (range 12-24 g; SD=3) and a mean length of 115 mm (range 104-131 mm; SD=7).

These fish were handled the same as the other fish in this study except that they were only permitted 2 hours to depth acclimate. In addition, only two depth-equivalent pressures, 20 ft and 40 ft, were tested.

3.0 Results

3.1 Mortality

The mortality of juvenile spring Chinook salmon exposed to simulated turbine passage varied depending on whether the fish was carrying a transmitter or not and the method of transmitter implantation (Table 3.1). The fish not implanted with a transmitter were less likely to die following exposure to simulated turbine passage (STP) than fish that were gastrically implanted with radio transmitters ($P=0.04$ for yearlings; $P<0.01$ for subyearlings). There was, however, no significant difference ($P=0.14$ for yearlings; $P=0.79$ for subyearlings) between survival of non-implanted fish and survival of fish surgically implanted with radio transmitters. Subyearling fish gastrically implanted with radio transmitters were significantly ($P=0.01$) more likely to die after exposure to STP than those that were surgically implanted with transmitters; however, there was no significant difference ($P=0.65$) for yearling fish.

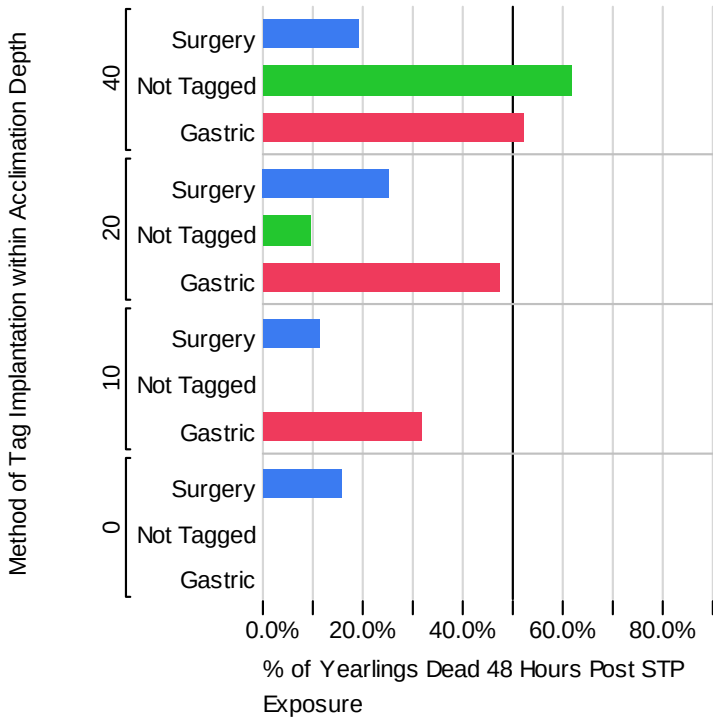
Mortality also varied with acclimation pressure but not by age class (Figure 3.1). Mortality increased significantly with the increased pressure at which the fish were acclimated ($P=0.01$ for yearlings; $P<0.0001$ for subyearlings). However, overall there was no significant ($P=0.5$) difference in mortality between yearling and subyearling fish based on acclimation pressure.

Mortality in fish not exposed to STP was generally low (Figure 3.2). None of the non-tagged yearling fish died and few of the non-tagged subyearling fish died.

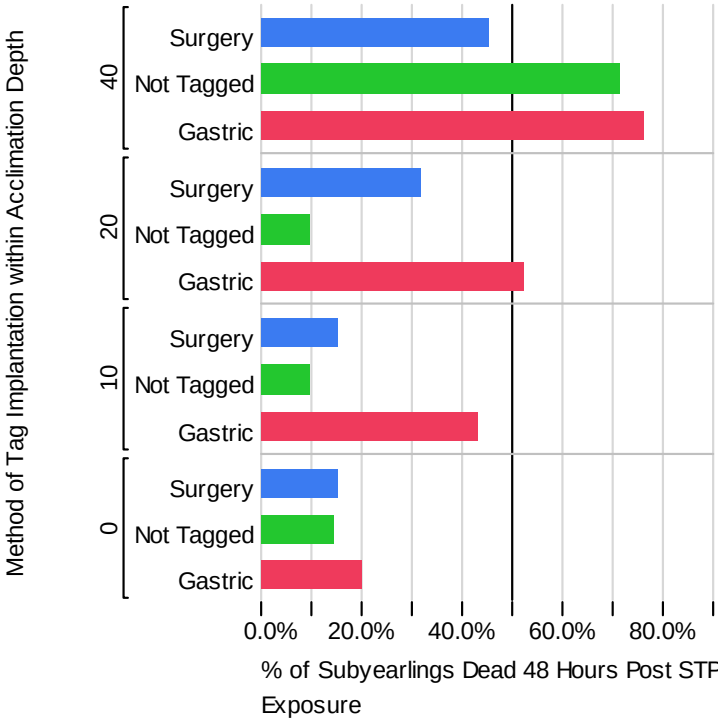
Table 3.1. Adverse Effects to Juvenile Spring Chinook Salmon Carrying a Surgical (S) or Gastric (G) Transmitter and Untagged (U) Fish that Were Exposed to Simulated Turbine Passage

	Yearling (1) vs Sub-Yearling (0)	Untagged vs Gastric		Untagged vs Surgical		Gastric vs Surgical		Damage Increasing with Acc. Pressure	
Age Class		1	0	1	0	1	0	1	0
Mortality	nsd	G > U	G > U	nsd	nsd	nsd	G > S	yes	Yes
Emboli In Gills	1<0	G > U	G > U	nsd	S > U	nsd	nsd	yes	Yes
Swim Bladder Rupture	nsd	G > U	G > U	S > U	nsd	nsd	G > S	yes	Yes
Fin Emboli	1<0	G > U	G > U	nsd	nsd	nsd	nsd	yes	Yes
<u>Disorientation</u>									
Post-STP	nsd	nsd	G > U	nsd	nsd	G > S	G > S	yes	Yes
Recovery	nsd	nsd	G > U	S > U	nsd	nsd	G > S	yes	Yes
Pre-Necropsy	nsd	G > U	nsd	nsd	S > U	nsd	S > G	yes	Yes

nsd = non significant difference

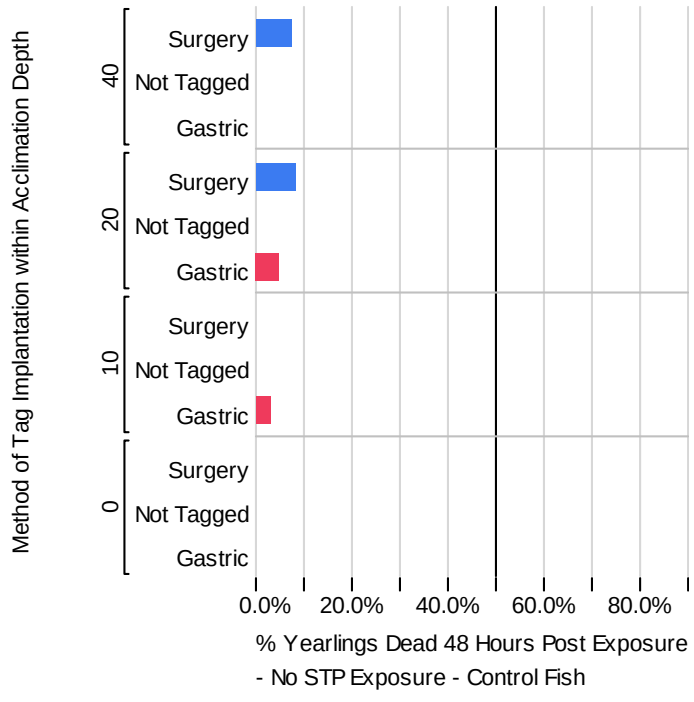


(a)



(b)

Figure 3.1. Percentage of Yearling (a) and Subyearling Chinook Salmon (b) that Died within 48 h after Simulated Turbine Passage



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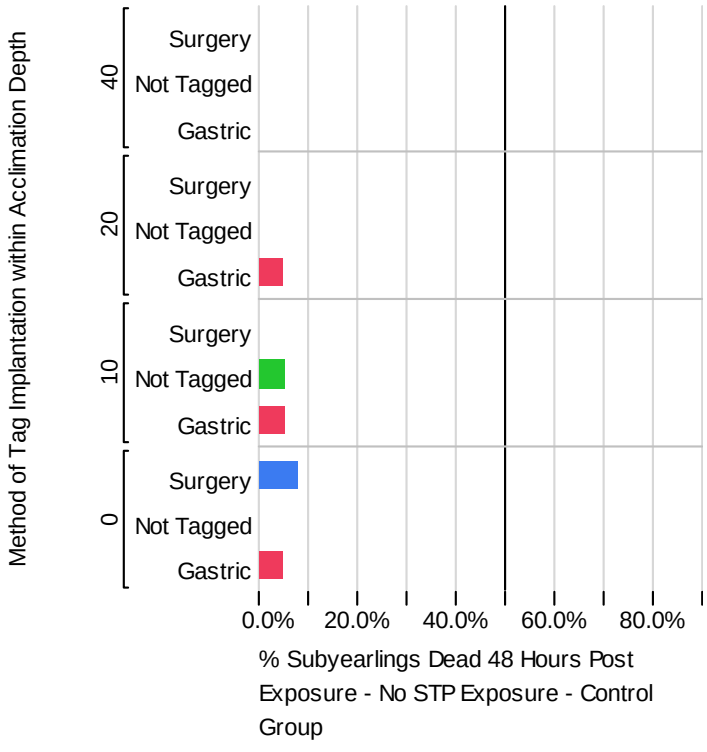


(b)

Figure 3.2. **P**ercentage of Yearling (a) and Subyearling Chinook Salmon (b) Not Exposed to a Simulated Turbine Passage (STP) that Died within 48 h after Tag Implantation

3.1.1 Emboli in Gills

A major cause of death was gas bubbles (emboli) in the gills (Figure 3.3). Their presence varied with acclimation depth, radio transmitter implantation technique, and age class of fish. Gill emboli were significantly (P=0.01) more common in subyearling than in yearling fish. In addition, the presence of emboli in the gills increased significantly (P<0.001) with acclimation depth for both yearling and subyearling fish. Gill embolisms were a relatively uncommon cause of death (less than 10% of fish) for fish depth acclimated at surface pressure. However, mortality due to gill emboli was more common when fish were acclimated at higher pressures (i.e., increased depth; Figure 3.4).

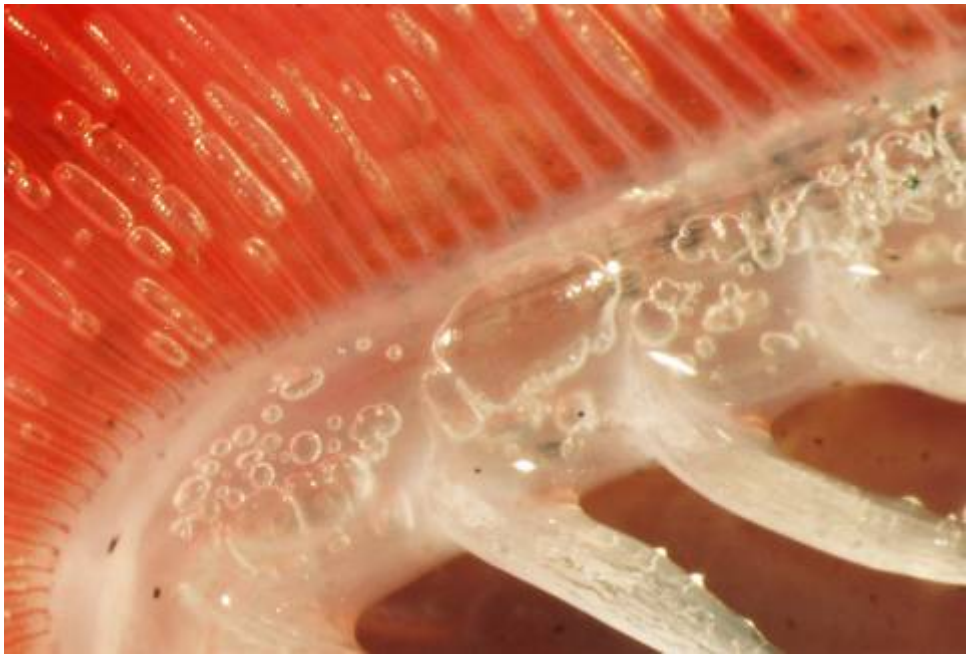
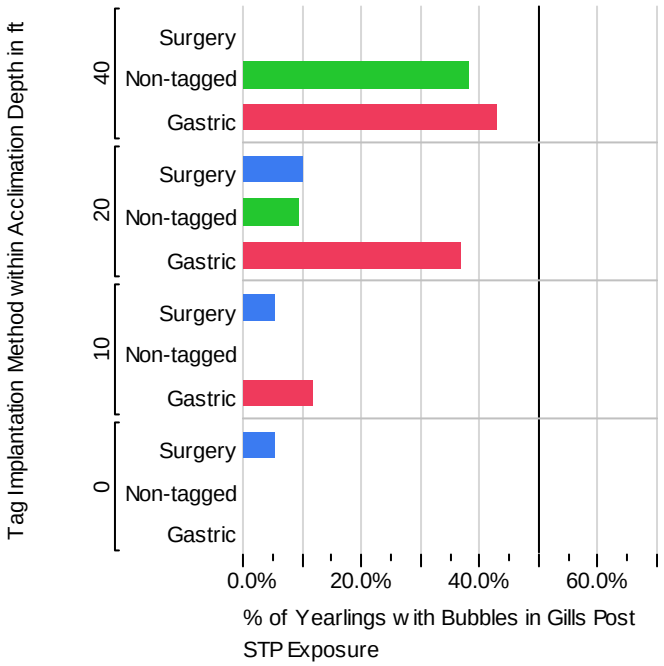
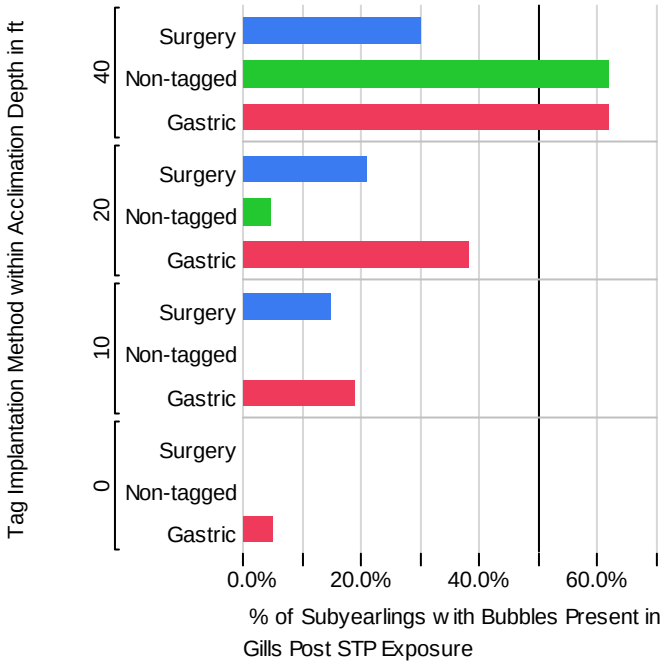


Figure 3.3. Emboli in the Gills of a Juvenile Chinook Salmon. Photo by Ralph Elston.

The presence of an implanted transmitter and the method of implantation influenced the presence of emboli in the gills. The presence of gill emboli was significantly more likely (P=0.02 for yearlings; P=0.003 for subyearlings) in fish gastrically implanted with a transmitter than in fish without transmitters (Figure 3.4). The presence of gill emboli was also significantly (P=0.04) more likely for yearling fish that were surgically implanted with a transmitter than for those that were not implanted; however, this difference was not significant for subyearling fish (P=0.96). There was a significantly higher incidence of gill emboli for fish gastrically implanted with transmitters than for fish surgically implanted with transmitters (P<0.001 for both yearling and subyearling fish) (Figure 3.4).



(a)



(b)

Figure 3.4. Percentage of Yearling (a) and Subyearling Chinook Salmon (b) with Gas Bubbles in their Gills following Exposure to Simulated Turbine Passage by Acclimation Depth

3.1.2 Damage to Spleens

Damage to the spleen of a fish may result in delayed mortality. The function of the spleen is to produce and store red blood cells – a critical life function. It was common for the spleen to be protruding

from the surgical incision of fish following STP (Figure 3.5). In a number of cases, the spleen of a fish with a surgically implanted transmitter would be found free in the chamber. Evidently the spleen had been pushed out of the surgical incision and pinched off when the incision closed. The spleen was protruding from 28% of subyearling and 9% of yearling fish that were surgically implanted with transmitters and then exposed to STP.

Figure 3.5. Spleen Protruding from the Surgical Incision of a Juvenile Chinook Salmon following Exposure to Simulated Turbine Passage

3.1.3 Stomach Eversion

Several fish had their stomachs turned inside out and partially extruded into their buccal cavity (i.e., stomach eversion) after exposure to STP (Figure 3.6). Most of these fish (4 of the 5) were gastrically implanted, half being yearling fish and half being subyearling fish. All of these fish died, and all had either internal hemorrhaging or emboli in addition to exhibiting stomach eversion.

Figure 3.6. Stomach Eversion in Juvenile Chinook Salmon Exposed to Simulated Turbine Passage

3.1.4 Ruptured Swim Bladders

The occurrence of ruptured swim bladders varied with acclimation depth and transmitter implantation technique (Figure 3.7). The incidence of swim bladder rupture increased significantly ($P<0.001$ for both yearlings and subyearlings) with depth-equivalent acclimation pressure. Few (<10%) non-tagged fish acclimated to surface pressure had ruptured swim bladders. However, as acclimation pressure increased, so did the occurrence of ruptured swim bladders for both implanted and non-implanted fish.

uvenile Chinook salmon that were not implanted with transmitters were significantly ($P=0.003$ for yearling; $P=0.001$ for subyearling) less likely to have ruptured swim bladders than those with gastrically implanted transmitters. Yearling Chinook salmon surgically implanted with transmitters had a significantly ($P=0.007$) higher occurrence of swim bladder rupture than non-implanted fish; however, there was no significant ($P=0.44$) difference for subyearling fish. For yearling Chinook salmon there was no significant ($P=0.82$) difference in the occurrence of swim bladder rupture between fish gastrically and surgically implanted with transmitters. Subyearling fish, however, were significantly ($P=0.02$) more prone to swim bladder rupture when gastrically implanted than when surgically implanted with transmitters.

For fish not exposed to STP (Figure 3.8), the occurrence of ruptured swim bladders was relatively uncommon. Some ruptured swim bladders in surgically implanted fish might have been caused by puncture during surgical implantation of transmitters. More detail on damage to fish attributed to transmitter implantation is given later in this report.

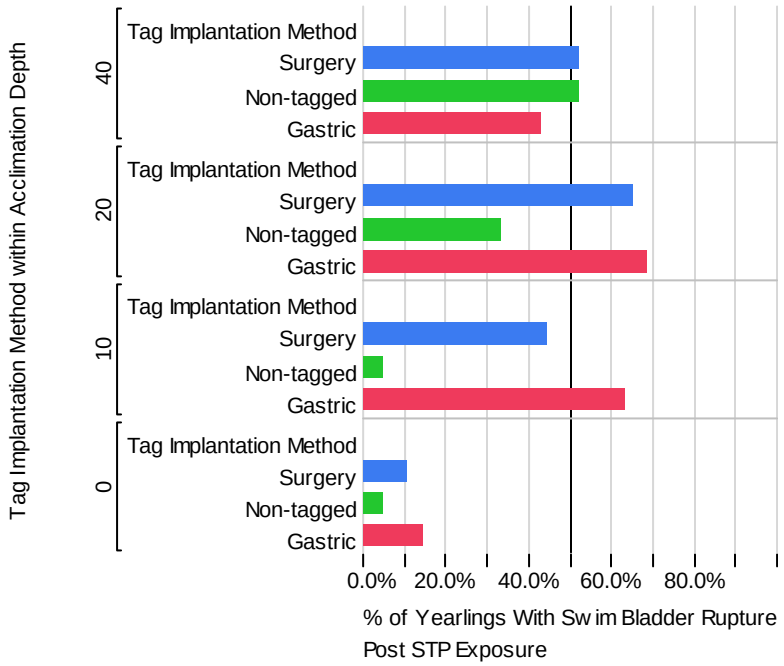
3.1.5 Embolisms in Fins

The presence of gas bubbles (embolisms) in the fins (Figure 3.9) varied with acclimation depth, implantation technique, and age of fish. The presence of gas bubbles in the fins increased significantly with the depth of acclimation ($P<0.001$ for both yearlings and subyearlings; Figure 3.10). Fin emboli were also significantly ($P=0.004$) more prevalent in subyearling fish than in yearling fish (Figure 3.10).

Gastric implantation was often associated with an increased occurrence of fin emboli. Subyearling Chinook salmon that were not implanted with transmitters were significantly ($P<0.001$) less likely to have fin embolisms than those with gastrically implanted transmitters; however, there was no significant difference ($P=0.13$) for yearling fish. Gastrically implanted yearling Chinook salmon had a significantly ($P=0.02$) higher occurrence of fin emboli than surgically tagged yearlings; however, this difference was not significant ($P=0.053$) in subyearling fish. There was also no significant difference in fin emboli occurrence between untagged and surgically tagged fish ($P=0.15$ for subyearling fish, $P=0.39$ for yearling fish).

3.1.6 Tag Expulsion

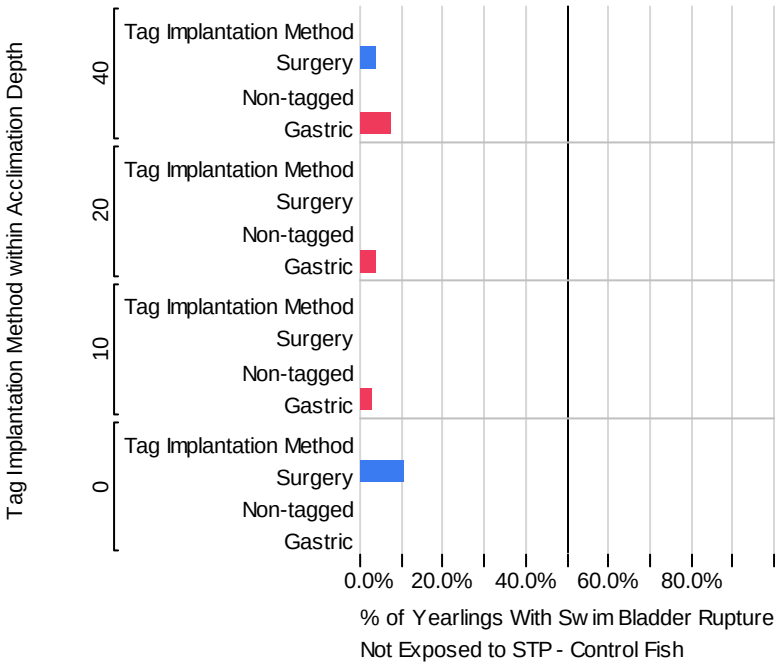
During the pressure spike, several fish expelled their gastrically implanted transmitter. This was seen in 2.4% (2 of 83) of subyearling and 3.8% (3 of 80) of yearling Chinook salmon. All of the fish that expelled their tags during the STP subsequently died of hemorrhaging or emboli in the gills.



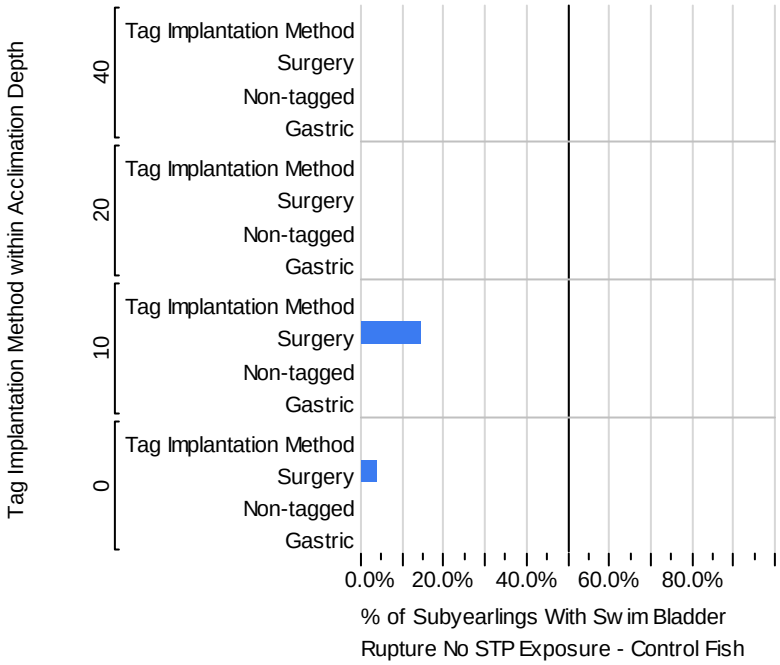
(a)

(b)

Figure 3.7. **P**ercentage of Yearling (a) and Subyearling Chinook Salmon (b) with Ruptured Swim Bladders following Exposure to Simulated Turbine Passage



(a)



(b)

Figure 3.8. **P**ercentage of Yearling (a) and Subyearling Chinook Salmon (b) with Ruptured Swim Bladders that Were not Exposed to Simulated Turbine Exposure

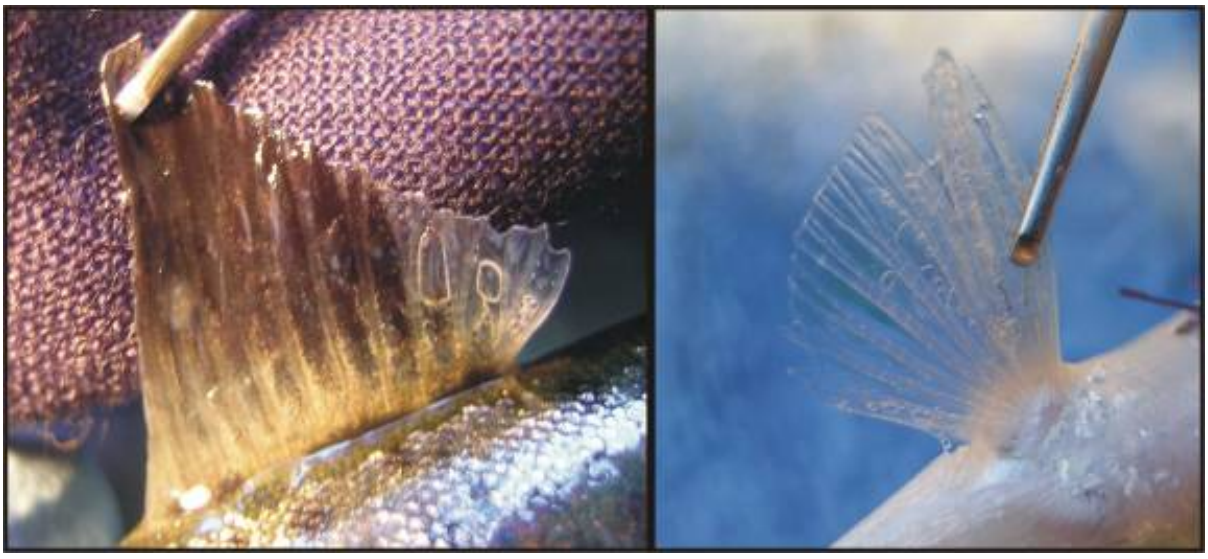


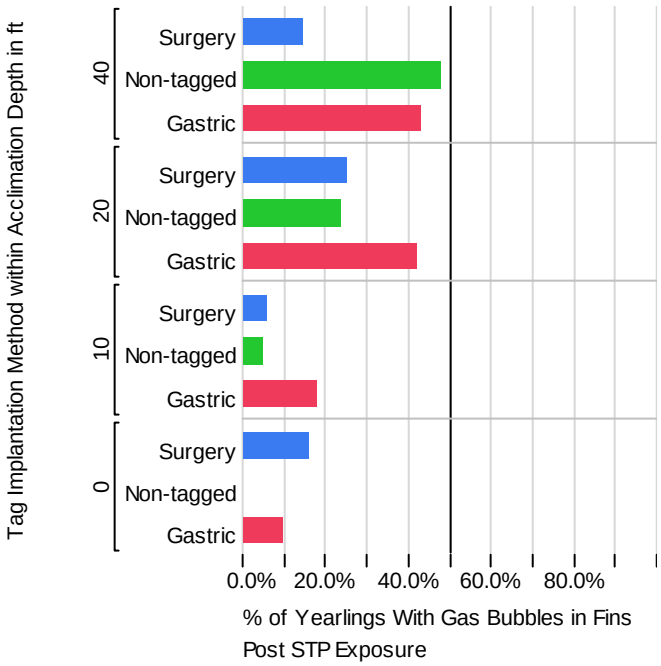
Figure 3.9. Emboli in the Dorsal Fin (left) and the Pelvic Fin (right panel) of Juvenile Chinook Salmon after Exposure to Simulated Turbine Passage

3.2 Histology

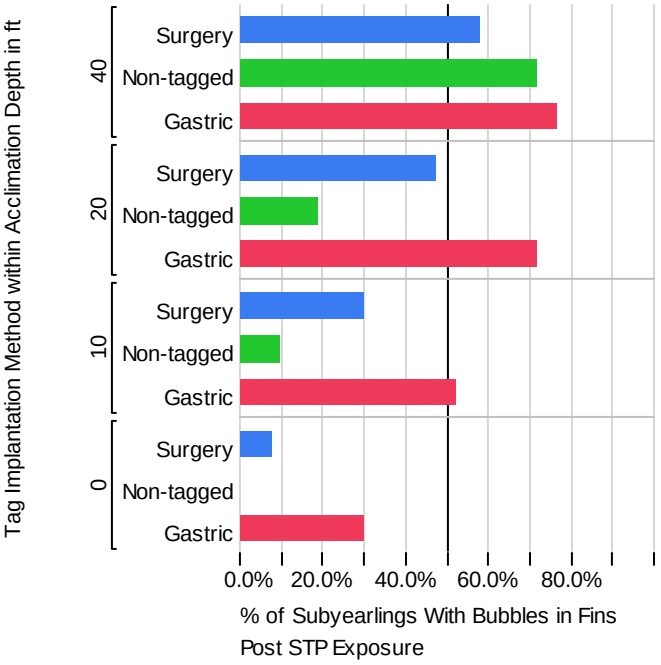
To identify the cause of death, we made histological examinations of juvenile Chinook salmon that died during and after STP. The most common lethal injury identified during histological examinations was internal hemorrhaging of the vascular system (Table 3.2). Hemorrhaging associated with the caudal vein was observed in 88% of 25 yearling fish and 93% of 41 subyearling fish examined using histological techniques. The caudal vein carries peripheral blood from the body musculature and certain organs back to the heart. A significant rupture in the caudal vein, as observed in these fish, would be expected to markedly reduce blood pressure and thus deprive the heart and organs supplied by arterial blood of adequate blood pressure. This condition is a reasonable interpretation of the cause of death of these fish.

In some cases, the rupture of the caudal vein was specifically observed. In other cases, the rupture was not in the section of fish examined but the hemorrhage was inferred by blood being found in the vicinity of the caudal vein. Hemorrhaging from the caudal vein often extended into the sub-peritoneal space between the top of the visceral cavity and the capsule of the kidney (between the peritoneal cavity lining and the kidney), into the swim bladder if it was ruptured, and into the peritoneal cavity (Figure 3.11 and Figure 3.12).

The next most common location for hemorrhage was within the pericardial sac (Figure 3.13). Pericardial hemorrhage was observed in 13% of fish (Table 3.2). This type of injury may be underestimated compared to rupturing of the caudal vein because it was observed in 81% (51 of 63) of fish that had saggital sections analyzed. Fish affected by this severe lesion had hemorrhage filling the pericardial cavity in all cases.



(a)



(b)

Figure 3.10. Percentage of Yearling (Top) and Subyearling Chinook Salmon (Bottom) with Fin Emboli after Exposure to Simulated Turbine Passage

Another type of injury that was observed only in saggital sections of fish was probable rupture of the ventral aorta. Rupture of the ventral aorta was believed to be the source of hemorrhaging seen in the connective tissue or thyroid near the ventral aorta. The connective tissue in the region of the ventral aorta in these cases contained diffuse and extensive hemorrhaging which, in one case, extended into the nearby thyroid follicles. These observations suggest possible rupture of the ventral aorta, but no rupture sites were found in the sections examined. The observed lesions might result from rupture of the bulbus arteriosus (one of the chambers of the heart; see Figure 3.14).

The least common serious injury observed was hemorrhage of the hepatic vein. This primary vein in the liver was found to be ruptured in one specimen.

Table 3.2. Relative Occurrence (% occurrence) of Four Different Types of Internal Hemorrhaging Observed in Yearling and Sub-Yearling Chinook Salmon during Histological Examination

	occurrence (%)		
Rupture	Yearling	Subyearling	combined
Caudal vein	22 (88)	41 (93)	63 (91)
Pericardial	2 (8)	7 (16)	9 (13)
Ventral aorta	1 (4)	4 (9)	5 (7)
Hepatic vein	0	1 (2)	1 (1)

Figure 3.11. Hemorrhaging Resulting from Rupturing of the Caudal Vein in a Juvenile Chinook Salmon Exposed to Simulated Turbine Passage. Photo by Ralph Elston.

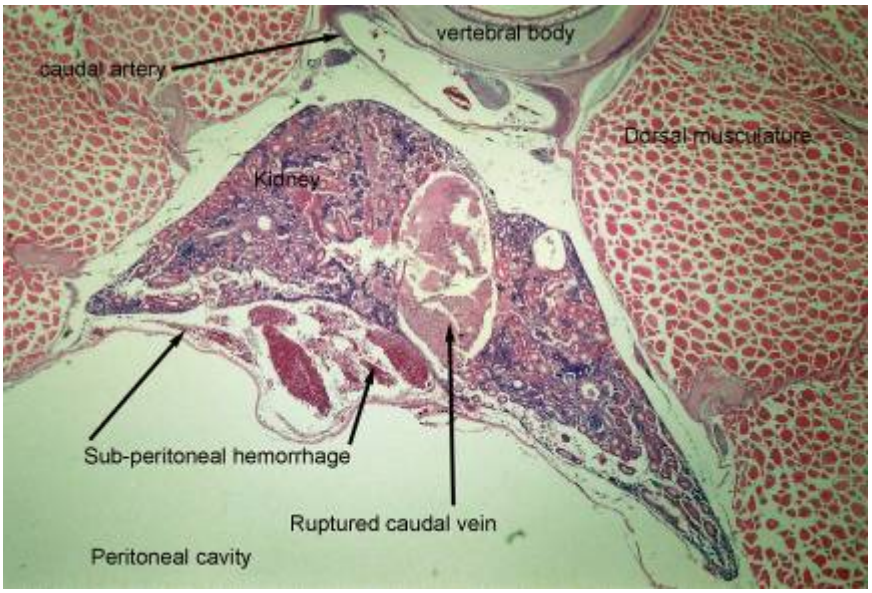


Figure 3.12. Hemorrhaging Resulting from Rupturing of the Caudal Vein in a Juvenile Chinook Salmon Exposed to Simulated Turbine Passage. Photo by Ralph Elston.

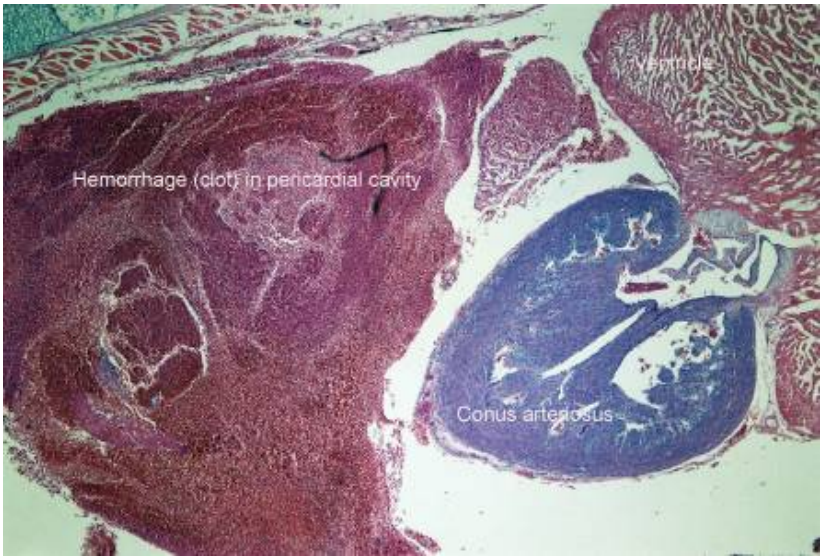


Figure 3.13. Hemorrhaging within Pericardial Cavity of a Chinook Salmon following Exposure to Simulated Turbine Passage. Photo by Ralph Elston.

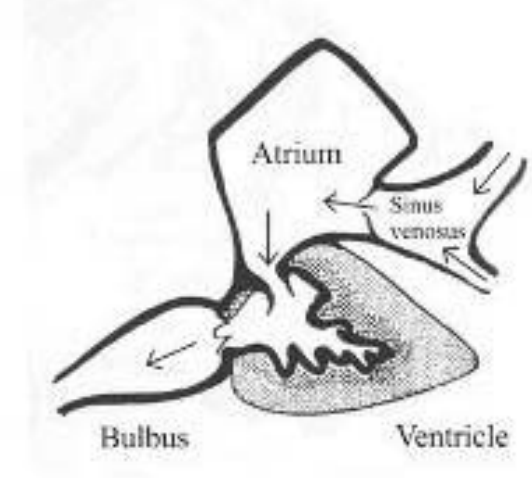


Figure 3.14. The Four Chambers of the Heart of a Salmon Contained within the Pericardial Sac: the sinus venosus, the atrium, the ventricle, and the bulbus arteriosus (from Hoar and Randall 1970)

3.3 Relationship between Buoyancy and Mortality

The buoyancy of juvenile Chinook salmon was estimated by visual observation of test fish behavior prior to exposure to STP. Observations of the state of buoyancy were made on 466 fish, of which 336 (72%) were designated neutrally buoyant (displacement equals mass), 106 (23%) negatively buoyant (displacement less than mass), and 24 (5%) positively buoyant (displacement more than mass) before being exposed to STP. There was no significant (P=0.30) difference in mortality between fish that were neutrally buoyant and those that were negatively buoyant. There was also no significant (P=0.57) difference in mortality between fish that were neutrally buoyant and those that were positively buoyant. This was also the case when fish were examined by age class (all P values >0.05).

3.4 Expulsion of Air during Simulated Turbine Passage

During simulated turbine passage, gas that is inside the fish (mainly in the swim bladder) can escape the fish's body via several routes. Gas can escape the swim bladder normally through the pneumatic duct and appear as bubbles exiting either the mouth or under the opercula (gill covers). Gas can exit by this route whether or not the swim bladder is ruptured. If the swim bladder is ruptured, gas can also exit through the incision made to surgically implant transmitters or through the vent. Of the 259 subyearling and 242 yearling Chinook salmon exposed to STP, 332 fish (~66%) were observed to expel air through one or a combination of the three possible routes during STP exposure (Table 3.3).

We tested the hypothesis that the expulsion of air during STP is related to juvenile Chinook salmon mortality. We first tested the hypothesis that fish that expelled air (regardless of site) during STP would have a decreased likelihood of mortality compared to fish that did not expel air. There was no difference in mortality associated with air expulsion; thus, we failed to accept our hypothesis since the likelihood of mortality was not significantly (P=0.11) higher for fish that did not expel air than for those that did. When divided by year class, this was also the case for both subyearling (P=0.08) and yearling fish (P=0.81).

Table 3.3. The Number of Fish that Expelled Gas through Three Difference Routes (pneumatic duct (PD), vent, or surgical incision, or a combination of those three routes). The percentage of fish from each category that had ruptured swim bladders is also shown.

Location of expulsion	N	Ruptured swimbladder (%)
PD only	206	18
Vent only	34	41
Incision only	28	32
PD and Vent	29	24
PD and Incision	31	13
Vent and Incision	3	100
PD, Vent and Incision	1	100
Total expelling gas	332	23

We next addressed the influence of location of air expulsion, expecting mortality to be higher among fish where air was expelled through the vent or surgical incision, thus indicating that the swim bladder was ruptured. This hypothesis was supported by our findings, since fish that only expelled air through the vent or the surgical incision, or in combination of these two, had a significantly higher mortality (P=0.04) than fish that expelled air from only the pneumatic duct.

We also tested the relationship between gas expulsion site and how mortality varied with year class. Yearling fish mortality was influenced more by the site of gas expulsion than was subyearling mortality. Yearling fish that only expelled air through the vent and/or the surgical incision had significantly (P=0.02) higher mortality than fish that expelled air only from the pneumatic duct. Subyearling fish that only expelled air through the vent and/or the surgical incision did not have significantly (P=0.59) higher mortality than fish that expelled air only from the pneumatic duct.

To refine the above analysis, we built a model to compare the mortality of fish that only expelled gas through the surgical wound to those that expelled air through the pneumatic duct. Mortality was significantly (P=0.007) higher for fish that expelled gas through the surgical incision than for fish that expelled gas only through the pneumatic duct. When analyzed separately, mortality was significantly (P=0.03) higher for subyearling fish when they expelled gas from the surgical wound than when they expelled from the pneumatic duct. However, there was only a marginal significance (P=0.08) in the difference for yearling fish.

We also hypothesized that fish that expelled air through the vent would have higher mortality than those that expelled air through the pneumatic duct. We failed to reject the null hypothesis of no significant difference (P=0.98) in mortality between the two groups. This was also the case when broken out by age class (yearling P=0.53; subyearling P=0.48).

We also wanted to examine the difference in mortality between fish that expelled gas from the vent and fish that expelled gas from the surgical incision. However, there were not enough data to perform this analysis.

3.5 Loss of Equilibrium

3.5.1 Directly after STP

Observations of the occurrence of a loss of equilibrium (LOE) were completed at three different times: 1) immediately post-STP while fish were still in the hyperbaric chamber, 2) two hours post-STP following transfer of test fish to holding aquaria, and 3) 48 hours post STP immediately prior to necropsy for all surviving test fish.

Immediately post-STP, LOE was markedly higher for fish acclimated at the 40-ft depth (Figure 3.15). There was a significant increase in the LOE with increasing depth of acclimation ($P<0.001$) for both yearling and subyearling fish. However, there was no significant ($P=0.71$) difference in LOE between yearling and subyearling fish.

The implantation of a transmitter into a fish and the implantation technique also influenced the equilibrium of the fish immediately post STP. LOE was significantly less likely ($P=0.01$ for yearling fish, $p=0.02$ for subyearling fish) in fish surgically implanted with transmitters than in those gastrically implanted with transmitters. Subyearling fish gastrically implanted were also significantly ($P=0.02$) more likely to exhibit LOE than non-implanted fish; however, there was only marginal significance ($P=0.08$) in the difference among yearling fish. There was also no significant difference ($P=0.26$ for yearling, $P=0.97$ for subyearling) in the occurrence of LOE between non-implanted and surgically implanted fish.

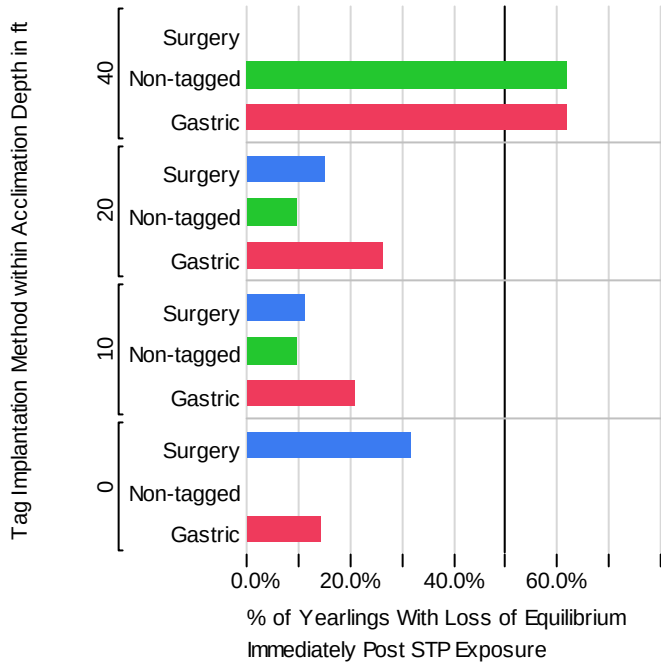
3.5.2 During Recovery

During the first two hours of recovery after fish were removed from the test chamber, LOE was influenced by acclimation depth and presence of a transmitter, but not fish age (Figure 3.16). There was a significant increase in LOE occurrence with increasing acclimation depth ($P<0.001$) for both yearling and subyearling fish. However, there was no significant ($P=0.11$) difference in LOE occurrence rate between yearling and subyearling fish.

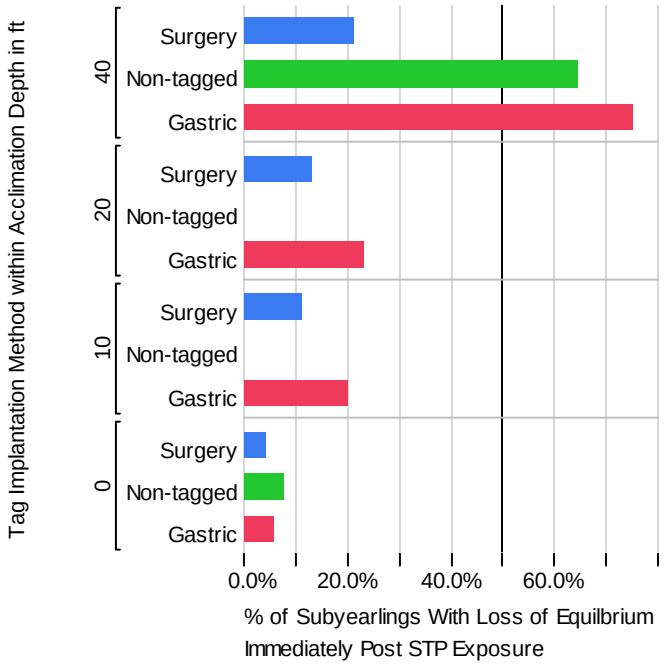
The presence of a transmitter and implantation method also influenced the equilibrium of test fish. LOE was significantly ($P=0.01$) more likely in subyearling fish gastrically implanted with transmitters than those not implanted with transmitters; there was no significant ($P=0.17$) difference for yearling fish. Surgically implanted yearling fish were also significantly ($P=0.02$) more likely to have a loss of equilibrium than non-implanted fish; however, there was no significant ($P=0.46$) difference among surgically and non-implanted subyearling fish. Gastrically implanted subyearling fish had a significantly ($P=0.003$) higher occurrence of a LOE than surgically implanted fish; however, there was no significant ($P=0.28$) difference between gastric and surgical implantation for yearling fish.

3.5.3 Pre-Necropsy

By the end of the 48-h holding period, the occurrence of LOE was still influenced by acclimation depth and the presence of a transmitter, but not by fish age (Figure 3.17). There was a significant increase in LOE with increasing acclimation depth ($P<0.001$ for yearling fish, $P=0.02$ for subyearling fish). However, there was no significant ($P=0.61$) difference in LOE occurrence between yearling and subyearling fish.



(a)

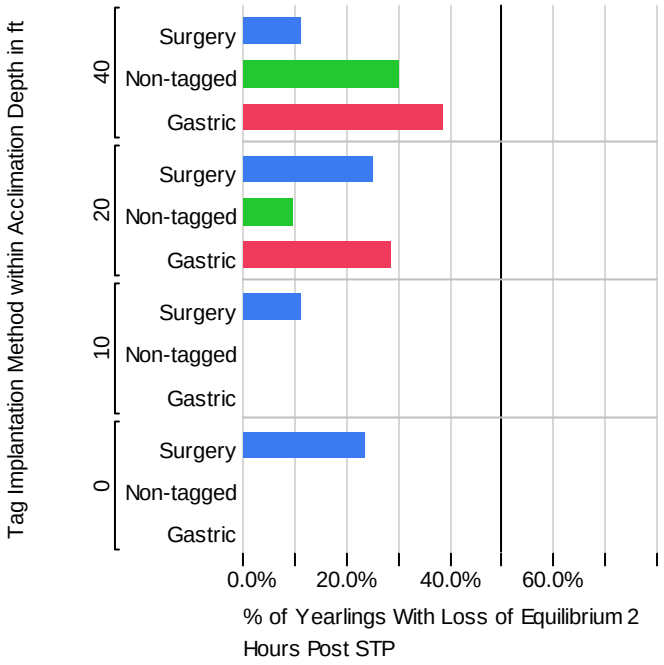


(b)

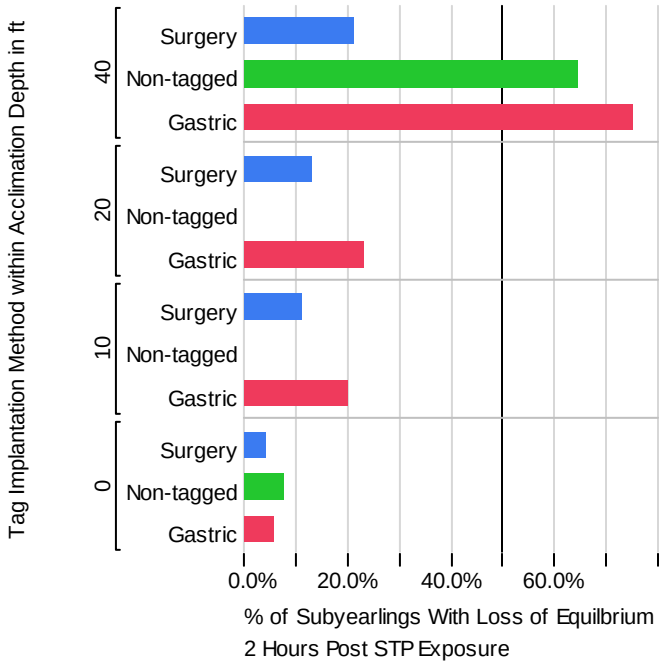
Figure 3.15. Percentage of Yearling (a) and Subyearling Chinook Salmon (b) Exhibiting LOE after STP. Observations were made after the pressure spike and before test fish were removed from the hyperbaric chamber.

The implantation of a transmitter into the fish and the implantation technique influenced LOE occurrence. LOE was significantly ($P<0.001$) less likely in yearling fish gastrically implanted with transmitters than in those not implanted with transmitters; there was no significant ($P=0.15$) difference for subyearling

fish. Surgically implanted subyearling fish were also significantly (P<0.001) more likely to show LOE than untagged fish; however, there was insufficient sample size to conduct this analysis for yearling fish. Surgically implanted subyearling fish had a significantly (P=0.01) higher occurrence of LOE than gastrically implanted fish; however, there was no significant (P=0.54) difference in yearling fish.

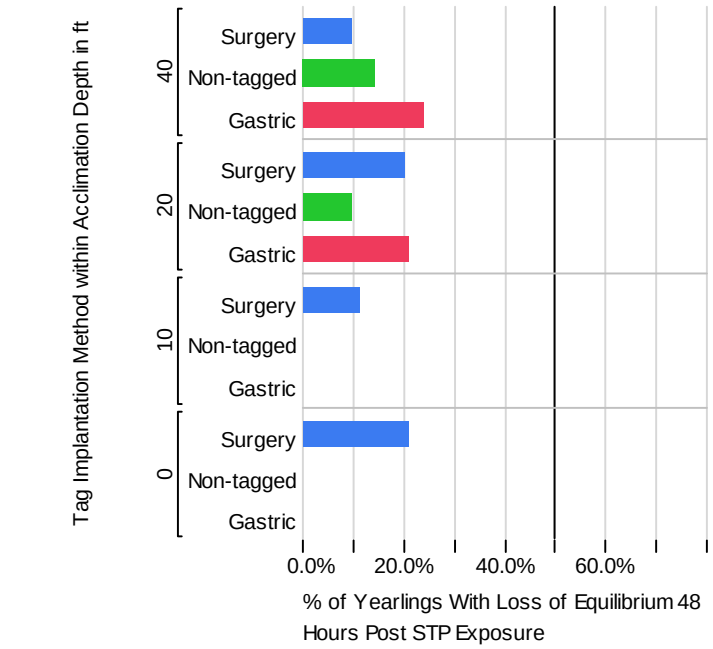


(a)

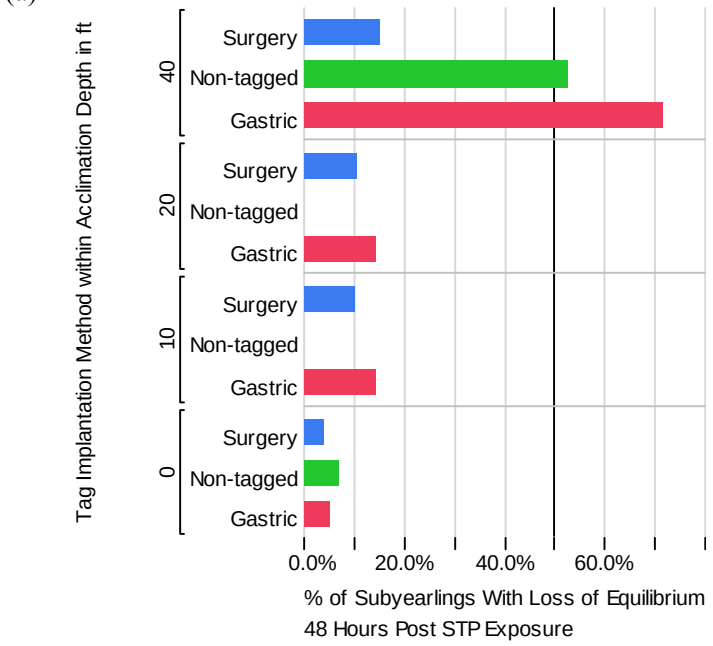


(b)

Figure 3.16. Percentage of Yearling (a) and Subyearling Chinook Salmon (b) 2 Hours Post-STP Exhibiting LOE. Observations were made during the 2-hour observation period after fish were removed from the hyperbaric chamber.



(a)



(b)

Figure 3.17. Percentage of Yearling (a) and Subyearling Chinook Salmon (b) Exhibiting LOE Forty-Eight-Hours Post-STP. Observations were made at the end of the 48-h holding period.

3.6 External Tagging Experiment

An experiment was conducted to determine the influence of STP on 61 subyearling Chinook salmon bearing a piece of lead with mass equal to a transmitter attached to the outside of their bodies. Similar to results of mortality for fish internally tagged, the mortality rate of the externally tagged subyearling Chinook salmon increased with acclimation depth. However, mortality was much higher than that of fish that were gastrically and surgically implanted prior to STP exposure (Figure 3.18).

3.7 Examination of Shorter Acclimation Periods

To determine whether differences in mortality or injury would occur when fish were depth acclimated for a shorter period (2 h rather than 16 to 24 h), four test runs were conducted in early August. There was no clear relationship between acclimation duration and mortality of gastrically implanted fish (Figure 3.19).

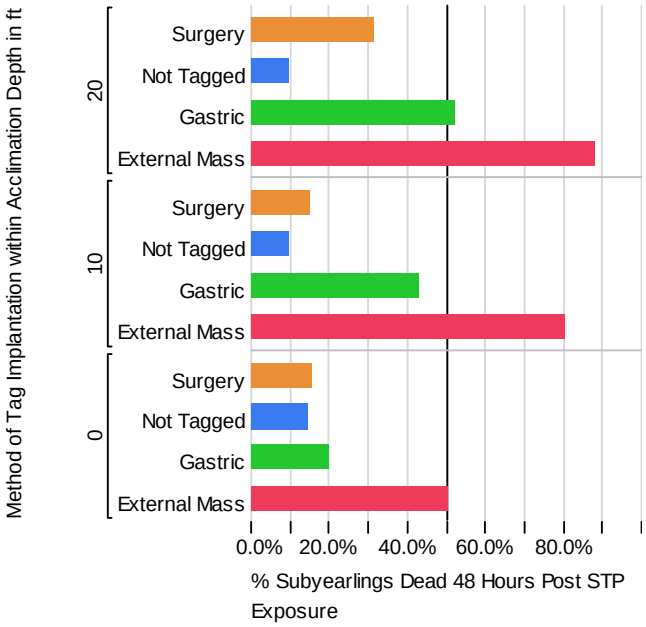


Figure 3.18. Percent of Subyearling Chinook Salmon Dead within 48 hrs after Exposure to the Simulated Turbine Passage with Four Different Tagging Treatments.

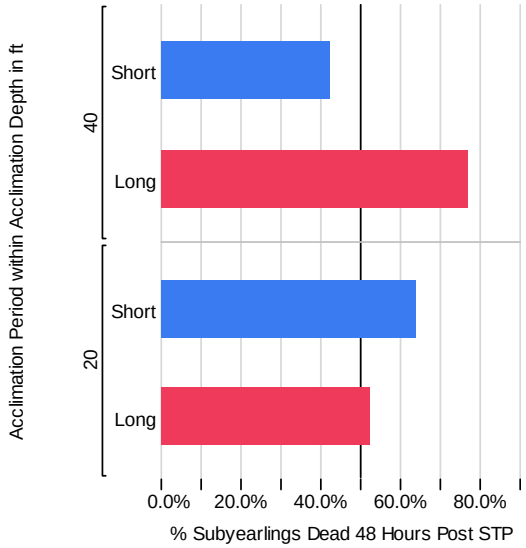


Figure 3.19. Total Percent of Subyearling Chinook Salmon Dead within 48 hrs after Exposure to Simulated Turbine Passage. Short = 2 h; Long = 16 h.

3.8 Evidence that Swim Bladder Ruptures Heal

Since damage to swim bladders was so prevalent during the study, an experiment was added to the study to determine if swim bladders of a common physostomous fish (juvenile rainbow trout) would heal after being damaged. Artificial swim bladder tears healed in all test fish within three weeks (Table 3.4). Approximately 30% of test fish had healed swim bladders within one week, and 90% had healed swim bladders within 2 weeks. All fish held for 3 to 4 weeks had healed swim bladders that held air.

Evidence of healing was thickening of tissue around the tear in the swim bladder (Figure 3.20) and the formation of scar tissue (Figure 3.21). Thickened tissue around the tear was only seen at day 7 of the experiment (Table 3.4). Visible scars were apparent through the end of the experiment.

In several instances, the healing swim bladder tear was found adhered to the interior wall of the body cavity (or mesentery) or fat deposits. These adhesions were seen during the first 2 weeks of the experiment, but not during the last 2 weeks (Table 3.5).

The state of inflation of swim bladders varied greatly over the course of the experiment (Table 3.6). After 1 week of healing, only half of the fish had any gas in their swim bladder. At this point, some fish still had a visible tear (opening) in the swim bladder, but still had some gas in the swim bladder. During the last 3 weeks of the experiment, all fish had gas in their swim bladder; however, none appeared to be fully inflated (although this is a subjective measure).

Table 3.4. Swim Bladder Healing Status of Juvenile Rainbow Trout in Percent over the Course of the Study

			Swimbladder healing status (%)		
Week	Treatment	N	Visible opening	Thickening around opening	Visible scar
1	Punctured	10	70	40	0
2	Punctured	10	0	0	0
3	Punctured	10	0	0	60
4	Punctured	10	0	0	10
4	control	10	0	0	0

Table 3.5. Swim Bladder Wound Adhesions to the Body Wall or Fat Deposits Observed in Juvenile Rainbow Trout

			Adhesion frequency (%)	
Week	Treatment	N	To body wall	To fat deposit
1	Punctured	10	10	10
2	Punctured	10	0	30
3	Punctured	10	0	0
4	Punctured	10	0	0
4	Control	10	0	0

Table 3.6. Status of Swim Bladder (SB) of Juvenile Rainbow Trout in Percent over the Course of the Study (N/A = non applicable)

			Swimbladder status (%)				
Week	Treatment	N	Healed SB	Air in SB	Fully inflated SB	Partially inflated SB	Fully deflated SB
1	Punctured	10	30	50	30	20	50
2	Punctured	10	90	100	80	20	0
3	Punctured	10	100	100	40	60	0
4	Punctured	10	100	100	70	30	0
4	control	10	N/A	100	100	0	0

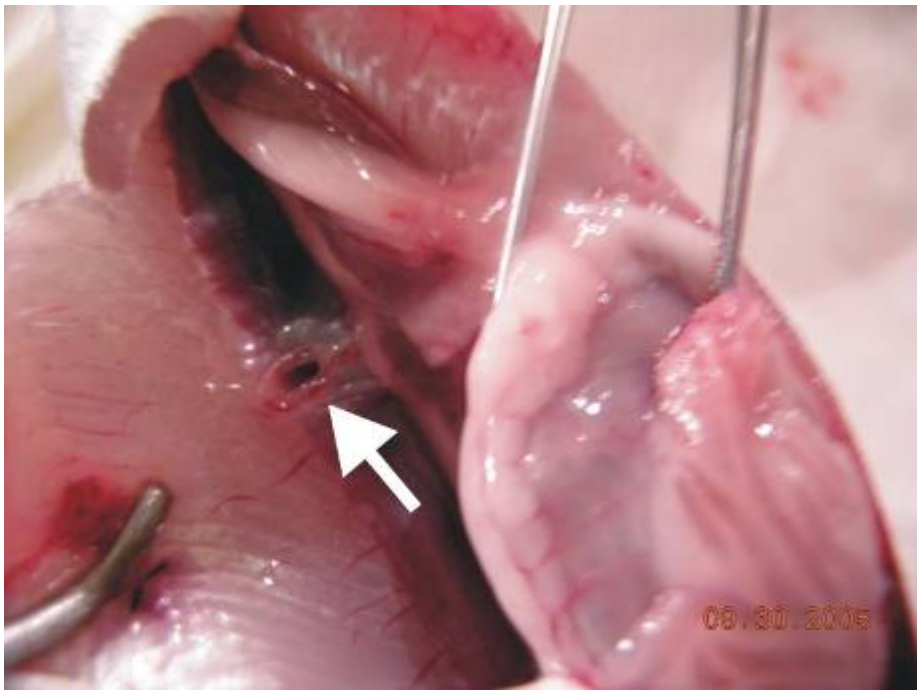


Figure 3.20. Swim Bladder of a Juvenile Rainbow Trout with Puncture Location Visible. Thickened tissue and redness is visible at the site of puncture.

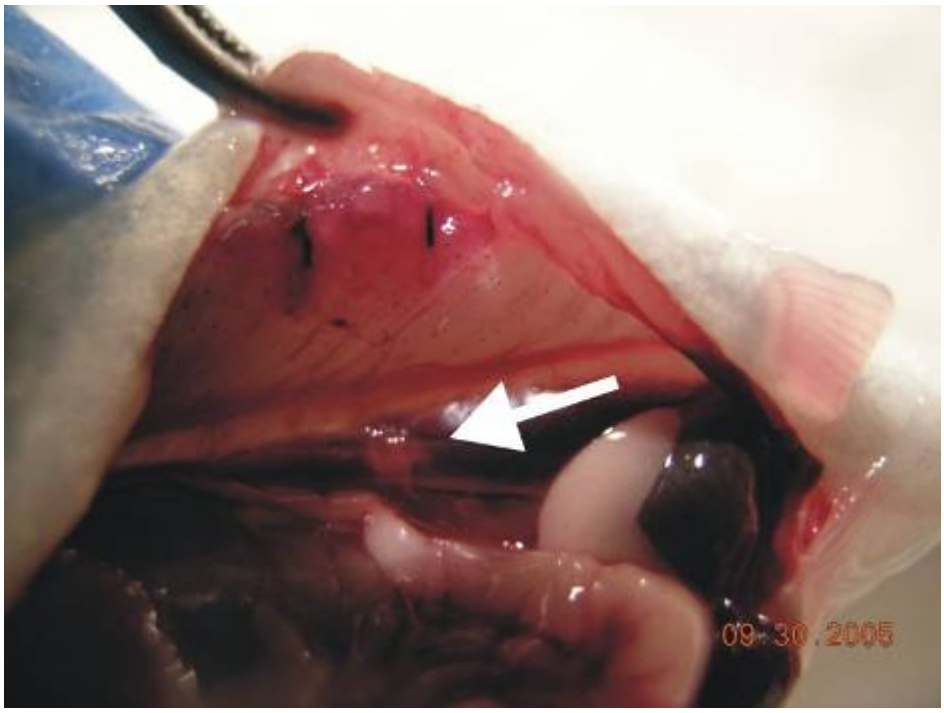


Figure 3.21. Scarring on the Swim Bladder of a Juvenile Rainbow Trout

3.9 Damage to Fish Due to Surgical Implantation

Several injuries believed to be caused by surgery during transmitter implantation were observed in the 406 fish surgically implanted for this study. The most common injuries observed were severing or cuts of the intestine caused when the antenna was routed through the fish's abdomen and out its body wall. This occurred in 10 fish (2.5%). The next most common injury was a puncturing of the swim bladder, which was observed in six fish (1.5%).

4.0 Discussion

The injuries and mortality of juvenile Chinook salmon observed in this study are the result of barotrauma caused by decompression. Barotrauma is defined as physical injury or death caused by change in pressure. Barotrauma is the biological consequence of physical changes in the volume and state of gas within an organism's bodily fluids, tissues, and organs. The barotrauma injuries and causes of death observed in this study are the result of the responses of biological processes to changes in the state of gas in response to changes in external pressure. These changes are the volume of gas bodies (swim bladder and bubbles) in tissues, organs, and fluids, and the solubility of gas in solution in bodily fluids.

Decompressive barotrauma can be caused by an increase in the pressure on organs within a fish's abdominal cavity when the swim bladder responds to decreasing external pressure by increasing in volume. Barotrauma can also occur when gas in solution in blood and tissues leaves solution as external pressure decreases and gas solubility also decreases. Gas released from solution increases the volume of blood which, in turn, increases the internal pressure in vessels, increasing risk of mechanical injury to vessels. Free gas forms bubbles that can also occlude vessels and be carried into organs with blood, thereby disrupting the normal function of organs such as the gills and heart. Usually increases in external pressure on organs caused by swim bladder expansion, increase in the number of bubbles in the blood, and increases in vascular pressure occur simultaneously. The severity of resulting barotrauma is then a function of the relative change in pressure (i.e., the ratio of initial and final pressures), other features of the exposure event, and the physiological condition of the fish (e.g., acclimation depth) at exposure to the change in pressure.

Physiological condition is the sum of a large number of factors which include the species and age of fish complicated by their acclimation history to pressure and water quality factors prior to exposure. There are, of course, many other factors that may influence the response of fish to changes in pressure; water temperature and the general health of the fish are also important factors.

There is a very high degree of variability in biological response to changes in pressure between individual fish with seemingly identical physiological states at exposure. The injury and mortality processes are equally complex. An example is heart failure resulting from accumulation of bubbles in the chambers of a fish's heart formed by gas driven from solution in the blood and carried into the heart by blood flow. Because of the strong gradients in physical processes that develop in response to changes in pressure, the physical events that cause barotrauma are essentially instantaneous in effect. While some biological responses can be equally sudden, such as immediate death resulting from heart failure due to much air in the chambers of the heart, others are delayed as in the case of a fish that is slowly internally bleeding to death from a ruptured vein. It is difficult to assess the risk to survival of other injuries such as stunning and disorientation, a relatively common occurrence for fish exposed to STP.

The chemical and physical processes causing barotrauma damage (i.e., gas embolism, hemorrhaging) occur during the rapid decompression of STP when both the fish tissues and surrounding water, saturated with gas at the level in the surrounding water at ambient pressure and temperature, become supersaturated during decompression (Bishai 1961; Beyer et al. 1976). Supersaturation leads to the formation of bubbles

in the blood and tissues (emboli) and the presence of these bubbles increases the volume and decreases the density of blood in the vascular system and organs such as the heart until ruptures occur (i.e., hemorrhaging), which can lead to massive blood loss and death. Concurrently, hyperinflation of the swim bladder is responsible for compression-related injuries of internal organs and, if the swim bladder ruptures, the gas from the swim bladder may enter the vascular system causing embolism (Bishai 1961) or be retained in the body causing irresolvable buoyancy imbalance.

This research has illustrated that the amount and severity of barotrauma and likelihood of direct mortality that occurs when juvenile Chinook salmon are exposed to rapid decompression by simulated turbine passage increases with the presence of a telemetry device in a fish's abdomen. This has significant implications for many survival and behavioral studies that use telemetry devices to estimate the survival and behavior of juvenile salmon as they pass through the Columbia River hydropower system. If injury and mortality are higher for fish bearing telemetry devices, then the survival estimates from the studies will underestimate survival and, in addition, it is unlikely that implanted fish will behave similar to fish without transmitters.

The presence of a telemetry device could increase the mortality and damage to fish in several ways. One way is damage due to amplification of swim bladder hyperinflation in response to rapid decompression. This happens in part because a transmitter increases the mass of the fish. Previous studies have shown that fish compensate (achieve neutral buoyancy) for the additional mass of a transmitter by increasing the volume of gas in their swim bladder, thereby increasing their displacement (Gallepp and Magnuson 1972; Anglea et al. 2003). As the fish pass through a turbine environment, they are initially exposed to increasing pressures as they pass through the turbine intake and approach the turbine distributor. Then, as the fish pass through the turbine runner they are exposed to lower pressures which, under worst-case conditions, can approach 0 psia (vapor pressure). If fish are carrying excess mass (a transmitter), they will increase swim bladder volume until their total body volume is sufficient to offset their body mass plus transmitter mass. Following Boyle's law, at rapid decompression, swim bladder hyperinflation will be increased in proportion to the additional swim bladder volume required to compensate for the additional transmitter mass. In general, it is expected that the change in swim bladder volume a fish experiences will be the ratio of acclimation pressure to turbine passage nadir pressure. This is based on the assumption that the internal swim bladder pressure required to achieve swim bladder volumes needed for neutral buoyancy displacements are equal to ambient pressures at acclimation depths. For example, a fish acclimated (neutrally buoyant) to near-surface depths (~15 psia) and exposed to a turbine passage pressure nadir of 5 psia will experience a swim bladder volume of three times what it had at its acclimation depth when it is exposed to the turbine passage nadir. Other studies have shown that decompression-driven swim bladder hyperinflation can lead to compression-related injuries of internal organs (Rummer and Bennett 2005) and increases in the occurrence and severity of embolisms (Bishai 1961). In our study, the presence of a transmitter resulted in more barotrauma damage to fish, particularly hemorrhaging, emboli, and other damage to internal organs. The extent of barotrauma observed varied with the method of transmitter implantation.

The presence of a gastrically implanted transmitter generally resulted in more damage and mortality than the presence of a surgically implanted transmitter. This may be due to the presence of a surgical incision in the fish, which could relieve some of the pressure on internal organs during swim bladder

expansion by permitting the abdominal volume of the fish to increase. Without an incision, the abdomen of a fish is surrounded by tissues which are essentially not extensible over the time of a rapid decompression event.

In addition to an increase in swim bladder volume, the presence of a surgical incision provides an outlet for gas that is released into a fish's abdominal cavity if the swim bladder is ruptured during rapid decompression. The release of gas from the abdomen decreases the internal pressure acting on a fish's organs. Other than release of gas from the vent, gastrically implanted fish do not have a way to relieve pressure on internal organs during swim bladder hyperinflation or to void gas that may enter the abdomen from a ruptured swim bladder.

The presence of a surgical incision could also decrease mortality since it permits greater abdominal expansion thereby decreasing the pressure acting on internal organs. Harvey (1963) examined the expansion of the swim bladder in juvenile sockeye salmon with and without a slit in the abdomen the length of the abdominal wall. He found that with the slit in the abdomen, the average expansion of the swim bladder of 10 sockeye salmon smolt was 289% (ranging from 242% to 311%) as compared to an expansion of 182% (ranging from 115% to 277%) in 10 sockeye salmon smolt without a slit in the abdomen. Although the slit that Harvey (1963) made was longer than the surgical incision used to implant transmitters in our study, Harvey's findings illustrate that the intact abdomen of a fish has a finite volume that limits the extent of swim bladder expansion and that an incision permits greater expansion. However, after the incision heals, it is assumed that the maximum abdominal volume of a fish would become normal and the mortality of surgically implanted fish might become more similar to that for gastrically implanted fish.

While the presence of a surgical incision allowed gas to vent following swim bladder rupture, which was correlated with a decrease in injury, fish that expelled gas through the surgical incision during STP still had higher mortality than fish that expelled gas through the pneumatic duct during STP. Venting of gas through a surgical incision could only occur after swim bladder rupture, which would, of necessity, follow swim bladder expansion and the possibility of compression of internal organs. It is likely that venting through the pneumatic duct occurs prior to over-expansion of the swim bladder in many cases, thereby preventing the occurrence of high abdominal pressures. Once swim bladder rupture occurs, the entry of gas into the body cavity could continue the physical processes, causing compression damage to organs and/or air could be forced into the vascular system resulting in the added complication of increased risk of emboli in tissues and organs. A frequently observed consequence of retention of gas in the body of a fish surviving swim bladder rupture was persistent positive buoyancy through the post-STP holding period. It is doubtful that fish in this condition would survive long in the wild.

The presence of gastrically implanted transmitters may lead to more damage and mortality than the presence of a surgically implanted transmitter due to its location within a fish's body. A surgically implanted transmitter generally sits farther back in the body cavity near the spleen, while a gastrically implanted transmitter is placed inside of the stomach of a fish. In this position the transmitter is anterior to the swim bladder. As a consequence, when the swim bladder expands, observations of tag expulsion indicate that swim bladder expansion may impulsively push the transmitter forward with considerable force toward the fish's mouth where it may hinder the expulsion of air through the pneumatic duct or

contribute to other injury processes. The pneumatic duct connects the swim bladder to the throat and the gastric transmitter may pinch off this duct during decompression, preventing gas from being expelled.

Differences in damage to fish were also dependent on factors other than tag implantation type, such as the size of the fish. The presence of emboli in both the gills and the fins was higher in the smaller subyearling fish than in the larger yearling fish. Beyer et al. (1976) suggested that small fish may be more influenced by rapid decompression than large fish due to such factors as a smaller lethal bubble size because of smaller vessel size, smaller unsaturated muscle mass, the lower gill surface-to-body-weight ratio, and relative cardiac output. Beyer et al. (1976) stated that smaller fish incur more damage due to the relative size of air bubbles in the body of the fish. Following decompression, theoretically, similar ranges of bubble sizes would occur in both small and large fish, assuming all sizes of fish were at equilibrium with external gas tension. However, since smaller fish have smaller blood vessels, it would be more likely that they would have more severe problems from flow blockages by bubbles than larger fish (Beyer et al. 1976). In addition, Tsvetkov et al. (1972) found that, during rapid decompression, fingerling and larvae of many physostomous species had more difficulty releasing gas from their swim bladders than did larger fish.

Other researchers have also observed an increase in mortality during decompression among smaller fish than larger fish. Beyer et al. (1976) held small (85-100 mm) and large (440-500 mm) coho salmon (*Oncorhynchus kisutch*) in a hyperbaric chamber without access to air at a pressure of 58 to 101 psi for various lengths of time (0.5 – 24 h) before rapidly decompressing them to surface pressure (14.7 psi). They found more damage to smaller fish after decompression than to larger fish. Contrary to their findings, Feathers and Knable (1983) did not find a difference in damage between two sizes (150-250 mm or 300-380 mm) of largemouth bass (*Micropterus salmoides),* a physoclistous species, following rapid decompression from 76.0 to 277.8 cm Hg (14.7 to 53.7 psi) to surface pressure.

Another factor that was associated with fish damage and mortality was the pressure at which acclimation occurred. Mortality and every type of behavioral and physical damage that was examined in this study increased with an increase in acclimation pressure. This trend was present for both yearling and subyearling fish. Higher mortality rates and increased occurrence and severity of damage resulted from increased amounts of gas in the swim bladder, tissues, and fluids of fish acclimated (neutrally buoyant and at gas equilibrium) to higher pressures. As the fish are subjected to the higher pressures present in deeper water, the fish's swim bladder compresses and the displacement of the fish decreases and the fish becomes negatively buoyant. Concurrently, the solubility of gas in the blood and other fluids increases, creating a gradient that causes gas to move into the fish's body from the surrounding water.

Fish compensate for negative buoyancy by increasing the number of molecules of gas within their swim bladder. Internal pressure within the swim bladder and swim bladder volume must be increased to, in turn, increase the volume of the fish to increase displacement and thereby buoyancy. Gas equilibrium between the fish's tissues and blood and the surrounding water is accomplished by the movement of gas molecules across the fish's gills and into solution in the fish's blood followed by transport to the fish's tissues. When the fish are rapidly decompressed during turbine passage, the gas in the swim bladder expands while that in the blood leaves solution resulting in compression, embolism, and hemorrhage barotrauma injuries. The greater the difference between acclimation and exposure pressures, all else

being equal, the greater the probability of injury and mortality as a result of the changes in volume and state of the gas within the fish's body.

During rapid decompression, other researchers have also seen more damage with increased acclimation pressure. Rummer and Bennett (2005) found that injuries to the swim bladder were most numerous and severe in the treatment group with the highest acclimation pressure relative to exposure pressure. They found that internal injuries showed clear patterns of progression and severity that were directly related to larger differences between acclimation and exposure pressure. They observed that as the pressure of acclimation increased, holding exposure pressure constant, displacement injuries were the first to occur as the expanding swim bladder increased in volume and applied pressure on internal organs and other structures. Higher acclimation to exposure ratios resulted in more severe compaction injuries as the swim bladder, confined by the body wall, exerted increasingly more pressure on internal organs "pinched" between the swim bladder and the fish's abdominal wall.

Beyer et al. (1976) also found increasing damage with increasing pressure of acclimation prior to rapid decompression. They placed juvenile coho salmon (85-100 mm, 7.5-9 g) in a hyperbaric chamber and exposed them to the pressures present at depths of 100, 133, 166, and 200 feet (58.1, 72.4, 86.7, and 101.4 psia) without access to air. Fish were exposed for varying periods of time (juveniles were held from 15 min to 24 h) and were decompressed to surface pressure at a rate of 100 ft/min (0.72 psi/s) before removal from the chamber. Damage to juvenile fish increased with acclimation pressure. Feathers and Knable (1983) also found an increase in mortality with increasing acclimation pressure for largemouth bass. They found that mortality was higher for largemouth bass that were acclimated to deeper depths (210.5 and 277.8 cm Hg; 40.7 and 53.7 psia) than for those at near-surface depths (76 cm Hg; 14.7 psi) prior to decompression in less than 1 minute to atmospheric pressure (76 cm Hg). They also found that delayed mortality was the major component of total mortality following depressurization from lower pressures (143.3 cm Hg; 27.7 psia) while initial mortality was the major component following decompression from higher pressures (210.5 and 277.8 cm Hg).

Damage to physostomous fish could also change with the amount of time that fish are allowed to acclimate (in the absence of total dissolved gas supersaturation [TDGS] condition) before rapid decompression. Prior to rapid depressurization, Beyer et al. (1976) acclimated juvenile coho salmon by holding them at higher pressure without access to air for periods of time varying from 15 min to 24 h. They found that the level of damage leveled off as acclimation time increased before decompression. Damage appeared to level off after 30 to 120 min of acclimation (in the absence of TDGS conditions). This may be why results of pilot research conducted during this study, which did not consider TDGS, did not find a significant difference in mortality between fish acclimated 16 to 24 h and those acclimated only 2 h.

Beyer et al. (1976) hypothesize that the period of time necessary to accommodate changes in gas solubility with depth and to equilibrate tissues should be relatively independent of the pressure; the period would be the same for a pressure increase of 1 atm as it would be for 5 atm. Therefore, they suggested that the 60 to 90 min acclimation time minimum for attaining maximum damage from decompression (with acclimation at 133 feet; 72.4 psi) indicates that at least the tissues of vital functional importance to the fish for immediate survival – heart, gills, vascular system, brain, and blood – had reached equilibrium with the external gas pressure within that time. They did not comment on the buoyancy state of their test

fish and did not consider TDGS exposure. D'Aoust and Smith (1974) used an acclimation time of 2 h before rapid decompression of juvenile coho salmon from 25.5 psia to 101 psia. They state that this period of time was adequate to saturate fish of this size by normal respiratory and tissue diffusion processes alone. These findings are important for estimating the minimum length of time that physostomous fish should be acclimated for blood and tissue gas equilibration before turbine survival tests.

Mortality was most often associated with hemorrhaging and emboli. Hemorrhaging was most often seen in the caudal vein. The next most common location was the pericardium, with hemorrhaging of the ventral aorta and the hepatic vein being less common. Hemorrhaging observed in the pericardial sac was similar to damage reported by Rummer and Bennett (2005) that resulted from rapid decompression of red snapper. They commonly found hemorrhaging and hematomas (a mass of clotted blood that forms in a tissue, organ, or body space as a result of a broken blood vessel) in the liver and loss of pericardial integrity or ruptures in the bulbous arteriosis (see Figure 3.15). Such active bleeding or structural damage to the heart would, in most cases, be immediately fatal (Nehoda et al. 2001). Following rapid decompression (with a difference between acclimation and exposure pressures as small as 1-1.5 atm; 14.7 – 22.0 psia), Tsvetkov et al. (1972) found hemorrhages in the peritoneum and kidneys of juvenile fish. Bishai (1961) suggested that, following rapid decompression, rupture of the swim bladder may result in bubbles of gas (from gas originating in the swim bladder) entering into broken blood vessels producing emboli in blood vessels and organs.

Emboli were an important cause of mortality observed during this study. The most obvious type of lethal emboli observed was in the gills. However, for several fish, during dissection, emboli were observed in the heart. The blood in the heart appeared very foamy because of the large amount of gas bubbles mixed in the blood, a condition that would likely lead to quick death. Similarly, Beyer et al. (1976) found that bubbles occluded blood vessels of juvenile coho salmon following rapid decompression. They also found gas bubbles in all chambers of the heart, but especially the atrium and the sinus venosus (see Figure 3.16). They observed that bubbles often completely occluded the bulbous arteriosis. This resulted in an expansion of the bulbous and the blocking of flow to the gills. The coronary arteries were also often filled with bubbles, blocking the supply of blood to the heart. Following rapid decompression, Beyer et al. (1976) also found emboli in the blood vessels of the liver, kidney, brain, gonad, and spleen. At higher acclimation pressures, they also observed emboli in the fat, gonadal material, and white muscle. D'Aoust and Smith (1974) also found emboli in the veins, muscle, and spinal cord of coho salmon (~6-10 cm long) following rapid decompression. Feathers and Knable (1983) often found large emboli in the cardinal and hepatic veins, sinus venosus, atrium, ventricle, and bulbus arteriosus following rapid decompression. They observed that these emboli appeared to displace most of the blood in these areas. Feathers and Knable (1983) also found emboli in the gills and in the brain following rapid decompression.

In our study, the presence of emboli in the fins increased with the ratio of acclimation to exposure pressure and was more frequent in smaller subyearling fish than in larger yearling fish. Beyer et al. (1976) also found emboli in the fins, tail, and lateral line of small coho salmon but observed that emboli were lacking or barely visible in larger (440-500 mm, 900-1400 g) coho salmon with the same

acclimation and exposure pressure treatments. Fin emboli, while most likely not fatal, are symptomatic of bubble formation elsewhere in the body of the fish.

Some of the behavior and damage we observed following STP, such as disorientation and damage to the spleen and swim bladder, would likely lead to delayed mortality. Essentially all fish exposed to STP were negatively buoyant when removed from the test chambers. It is expected that river-run fish would exit the turbine environment in the same condition.

During the post-exposure holding period, essentially all fish that did not have damaged swim bladders gulped air at the surface to refill their swim bladders and achieve neutral buoyancy. If river-run fish exhibited this same behavior following turbine passage, it is likely that time spent near the water surface could increase their exposure to predation by birds and fish (Reiman et al. 1991). Swim bladder rupture and inability to recover neutral buoyancy could lead to a loss of equilibrium, which likely makes fish more susceptible to predation.

Damage to or loss of the spleen would weaken a fish's immune system. This could lead to either direct or indirect mortality. Untreated lacerations of the spleen in vertebrates can result in instability of blood circulation, inflammation of the peritoneum, and septic conditions (Kuehnert 1993). Rummer and Bennett (2005) suggest that while more severe injuries seen during rapid decompression such as puncturing or rupturing of vital organs may be acutely lethal, less severe injuries such as compaction of organs may have lingering chronic lethal effects. Even minor insult, such as displacement or bruising, could potentially inhibit performance and, ultimately, survival (Rummer and Bennett 2005). They also suggest that following rapid decompression, mild gastrointestinal damage could have long-term effects that hinder fish performance and survival because many fish that were rapidly decompressed stopped eating after weeks of normal feeding behavior (prior to exposure) and apparently died from starvation.

This study did not find any difference in the rate of occurrence of swim bladder rupture between yearling and subyearling Chinook salmon following STP exposure. However, Tsvetkov et al. (1972) found that during rapid decompression, fingerling and larvae of physostomous fish had greater difficulty releasing gas from their bladder than larger, older fish. We did not test larvae or fingerling fish. If the findings of Tsvetkov et al. (1972) are applicable to Chinook salmon and other species in the Columbia River, we would expect a greater incidence of swim bladder rupture among smaller fish following turbine passage.

Our experiments were conducted with juvenile river-run Chinook salmon that were acclimated to the total pressure present in a range of 0 to 40 feet of water (14.7 to 32.0 psia). However, it is unclear if the Chinook salmon, a physostomous fish, can gulp enough air into its swim bladder at the surface of the water and keep it in its swim bladder to be neutrally buoyant at a depth of 40 ft. At 40 ft in fresh water, the absolute pressure would be 32 psia. Essentially no research has been conducted to determine the range of depths at which juvenile Chinook salmon and other species of salmonids can maintain neutral buoyancy given the constraint of having to fill their swim bladders by gulping air at the water surface. A crude estimate of the extensibility (maximum volume) of the swim bladder of physostomous salmonids can be attained from the work of Harvey (1963) on juvenile sockeye salmon. He examined the extensibility of their swim bladders in relation to vertical migration behavior. He decreased the pressure on surface-acclimated test fish until the pressure of the expanding gas in the swim bladder increased

enough so it escaped past the pneumatic duct. His goal was to determine the pressure at which the pneumatic duct could no longer hold gas in the swim bladder of surface-acclimated juvenile sockeye salmon, thereby obtaining an estimate of the maximum volumes of the swim bladders of test fish. He found that sockeye salmon smolt had a mean extensibility of 83% above the bladder volume at neutral buoyancy at near-surface pressure. Among the 10 sockeye salmon he examined, extensibility (the increase in swim bladder volume above what the fish maintained for neutral buoyancy at near surface pressure) ranged from 15% to 177%. However, his pressure reduction tests were conducted using fish that had been recently killed. It is not clear that this testing method accurately estimates the internal pressure threshold for gas release through the pneumatic duct.

The values of swim bladder extensibility obtained by Harvey (1963) can be used to get a crude estimate of the ability of a juvenile salmon to maintain neutral buoyancy at different depths. For a fish to attain neutral buoyancy at a certain depth it must gulp air at the surface into its swim bladder and swim down into the water until the increasing pressure compresses the fish, including the air in the swim bladder, to the point where the fish's displacement equals its excess mass. At this depth, the fish is neutrally buoyant. Using the mean value of swim bladder extensibility (83%) determined by Harvey (1963), an average physostomous fish could not gulp enough air at the surface and contain it in the swim bladder to become neutrally buoyant at depth greater than ~28 ft (Figure 4.1). If more air was gulped into the swim bladder at the surface, the pressure would be too high for the pneumatic duct to contain the gas. However, Harvey (1963) found a range of extensibility of 15% to 177%, which implies that some salmonids can gulp enough gas in their swim bladders to maintain neutral buoyancy at a depth greater than 50 ft while others would not be able to attain neutral buoyancy at depth much greater than a foot or so. Also, since the fish were dead when his experiments were conducted, and the pneumatic duct may have been more relaxed, fish may be able to attain neutral buoyancy at even greater depths. However, these data are pilot scale at best, sample sizes were very small, and the methods were never shown to be applicable to live fish. The bottom line is that the range of depths over which physostomous fish are able to attain neutral buoyancy is unknown.

Although the swim bladders of fish that are neutrally buoyant at greater depths will expand a greater amount than those of fish neutrally buoyant at shallower depths given the same exposure pressure, the amount of expansion can be quite large for surface-acclimated fish depending upon the ratio of acclimation and exposure pressures. A fish neutrally buoyant at surface pressure (14.7 psia) would experience an increase in swim bladder volume of almost 900% when exposed to a pressure decrease to 1.5 psia during simulated turbine passage (Table 4.1, Figure 4.2). Thus even fish neutrally buoyant at the surface may be subject to swim bladder rupture and compression injuries (although in our study it was observed that less than 10% of surface-acclimated test fish exposed to STP suffered ruptured swim bladders) and related disorientation.

We also conducted a pilot experiment to determine if externally attaching a transmitter to a fish led to lower mortality than either gastric or surgical implantation of transmitters. However, our results showed even more damage to fish bearing external transmitters than fish bearing either gastric or surgically implanted transmitters. Previous studies as well as ours clearly show that juvenile Chinook salmon achieve neutral buoyancy by compensating for the excess mass of internally implanted transmitters by increasing the volume of gas in their swim bladder (Gallepp and Magnuson 1972; Anglea et al. 2003) and

thereby their displacements. The reasons we observed higher mortality and injury rates for test fish bearing external transmitters than we observed for fish bearing internal transmitters are not known. We also observed that our test fish had considerable difficulty maintaining equilibrium during acclimation. Given the pilot scale of our experiment and other study factors, we cannot draw any conclusions at this time about the response of juvenile Chinook salmon to the presence of external transmitters.

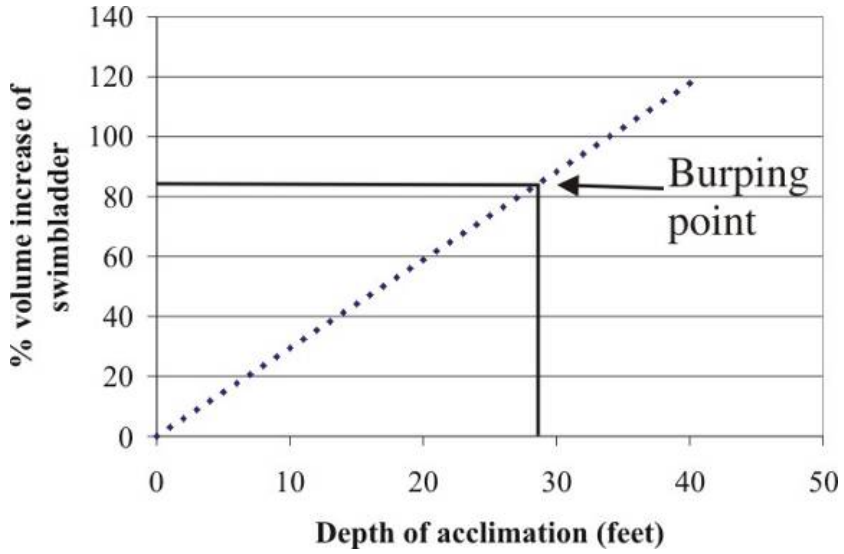


Figure 4.1. The Increase in Swim Bladder Volume of Fish Acclimated to Different Depths (neutrally buoyant at that depth) and then Brought to Surface Pressure. Harvey (1963) estimated that juvenile sockeye salmon could not increase swim bladder volume by gulping air at the surface more than a mean value of 83% above that required for neutral buoyancy at near-surface depths (range 15% – 177%) without expelling air from the swim bladder.

Table 4.1. The Percent Increase in Swim Bladder Volumes for Fish Neutrally Buoyant at the Pressure Present at 0 to 40 feet when Pressure is Decreased to Different Levels

Pressure decreased to (psia)	Depth of acclimation (feet)				
	0	10	20	30	40
	Percent increase in swimbladder volume				
14.7	0	29	59	88	118
3	390	534	679	823	967
1.5	880	1169	1457	1746	2034

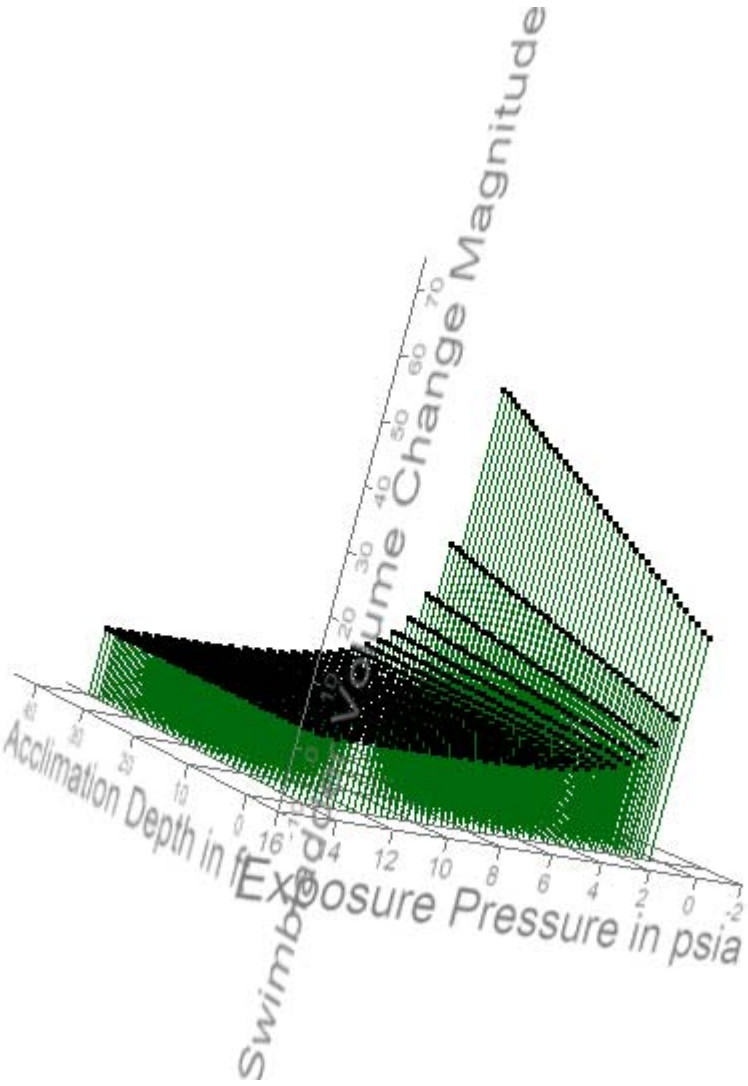


Figure 4.2. Relationship between Acclimation Depth, Exposure Pressure, and Expected Change in Swim Bladder Volume Using Boyle's Law

5.0 Conclusions and Recommendations

This research has made clear that exposure of juvenile Chinook salmon bearing radio telemetry transmitters to simulated turbine passage with a nadir of 1.5 to 3.0 psia may result in barotrauma resulting in immediate or delayed mortality. The study also identified sublethal barotrauma injuries that may increase susceptibility to predation in addition to both immediate and delayed mortality. The most common causes of immediate mortality were found to be hemorrhaging and emboli. Mortality and injury rates were observed to vary with transmitter implantation method. Gastric transmitter implantation resulted in higher rates of injury and mortality than surgical implantation. In addition, barotrauma injuries were more extensive for smaller subyearling Chinook salmon than for the larger yearlings. All observed types of barotrauma injuries and mortality rates increased with acclimation pressure for both age groups.

This study is the first to identify acclimation depth as an important variable affecting barotrauma injury and mortality resulting from rapid decompression for physostomous fish even though it has been long recognized as a factor for physoclistous fish. This study is also the first to examine the effects of the presence of internal radio transmitters on rapid decompression-induced barotrauma and it is the first study to include a method of implantation as a treatment factor for study of rapid decompression for physostomous fish.

The results of this study indicate that estimates of turbine passage survival for juvenile Chinook salmon obtained using radio telemetry devices may be biased. The presence of radio telemetry transmitters has been shown in this study to significantly affect barotrauma injury and mortality rates for fish exposed to pressure nadirs in the range from 1.5 to 3.0 psia. However, at this time it is not possible to extend our findings to the population of radio telemetry-tagged fish passing through turbines. Lacking is information about the portion of the turbine-passed population exposed to low nadirs or the effects of exposure to nadirs higher than 3 psia. Also needed are point estimates of effect levels for barotrauma injury and mortality over the range of nadir values likely to be experienced by fish passing through turbines.

Now that acclimation pressure has been identified as an important factor affecting the outcome of exposure to rapid decompression for physostomous fish, studies are needed to reconsider guidance previously provided to turbine manufacturers about criteria for exposure of physostomous fish to decompression during turbine passage. At this time the lack of this information prevents assessment of the likely effects of rapid decompression on the population of untagged physostomous fish passing through hydro turbines.

The high occurrence of sublethal effects such as swim bladder rupture and loss of equilibrium observed for treated fish indicates that exposure to very low nadirs during turbine passage may affect the behavior of transmitter-bearing fish for a significant period of time following exit from the turbine environment. Although not evaluated in our study, it is possible that these effects and others resulting from other types of sublethal injuries might increase the susceptibility of turbine-passed fish to downstream predation. While it is important to acknowledge the occurrence of significant sublethal injuries and to suggest their importance for survival of turbine-passed fish, it is also important to state

again that the results of this study cannot be extrapolated to the larger population of turbine-passed fish. The reason for this is that the pressure exposure simulated was the lowest pressure likely to be experienced by fish passing through a turbine and there is a lack of information about the proportion of fish passing through a turbine that are likely to be exposed to these low pressures.

Telemetry is a very important tool for assessment of the behavior and survival of juvenile salmonids migrating through the Federal Columbia River Power System. Further work is necessary to identify thresholds for the mass and volume (and other features) of implanted transmitters that will minimize the risk of barotrauma injury and death for fish passing through turbines. Large changes in pressure are also experienced by fish passing in tainter gate-controlled spill. Transmitters that would meet the rigorous requirements imposed by "no effect" criteria for pressure exposure might reduce the risk of injury from other exposures as well or permit a transmitter to be retained within the body of a fish over its life cycle without deleterious effects.

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