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Assessing barotrauma in neutrally and negatively buoyant juvenile salmonids exposed to simulated hydro-turbine passage using a mobile aquatic barotrauma laboratory

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ABSTRACT

Barotrauma-injuries sustained following rapid decompression occur in many different fisheries applications. Previous attempts to quantify barotrauma in fish have been limited by the functionality of hypo/hyperbaric systems. Further, field studies often are confounded by covariates. The mobile aquatic barotrauma laboratory (MABL) was designed to address these limitations. Specifically, this testing facility allows the user to evaluate similar complex pressure scenarios to which migrating juvenile salmonids are exposed following turbine or spillway passage. In this paper, we describe the MABL and present a case study in which negative and neutrally buoyant juvenile Chinook salmon were exposed to simulated hydro-turbine passage (STP). The severity of the decompression profile and the fish's ability to gain neutral buoyancy were used as predictor variables. We determined that following STP, fish that achieved neutral buoyancy during a 16-h acclimation period had a greater risk of mortality and injury (gill emboli, swim bladder rupture, and internal hemorrhaging) than negatively buoyant conspecifics. This research solidifies the need to allow fish to become neutrally buoyant when assessing barotrauma and mortality in field and laboratory applications. Future research examining injury and mortality of turbine-passed fish needs to consider the fish's buoyancy to more appropriately evaluate these endpoints.

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1. Introduction

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

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

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concern in catch-and-release angling (Rummer and Bennett, 2005; Gravel and Cooke, 2008; Wilde, 2009), prompting many fisheries scientists to investigate management strategies that would address and minimize the negative effects caused by barotrauma.

Given the importance of barotrauma to fisheries ecology, there is a need to systematically test the variables responsible for dictating the incidence of barotrauma and the resulting injury. Historically, field covariates (e.g. variation in water temperature, total dissolved gas, rate of pressure change, etc.) have prevented researchers from examining the specific pressure profiles of exposure. In the laboratory, the usefulness of simple hypo- (decreasing pressure) and hyperbaric (increasing pressure) chambers for conducting barotrauma research have been limited for several reasons. Some systems have been able to provide only static water scenarios; some have provided only constant or limited levels of total dissolved gas, while others performed only simplistic pressure exposure profiles (e.g., Harvey, 1963). Thus, experiments performed in these chambers may have limited biological or environmental relevance.

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

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

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

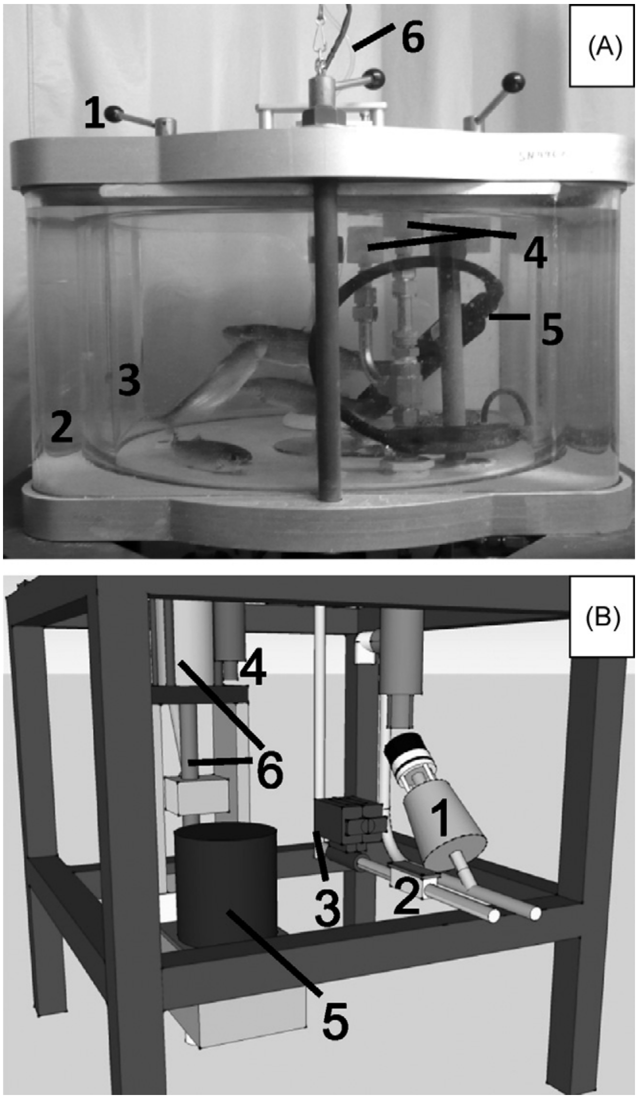

Fig. 1. (A) One of the hypo/hyperbaric chambers of the MABL system. The chamber hatch is tightly closed with four dogs (1). A lens (2) surrounds the inner chamber (3) to allow for undistorted viewing. Float switches (4) maintain water level and an in-chamber sensor (5) monitor TDG and temperature. Conditions are relayed by the system GUI (not pictured). A bubble at the top of the chamber is maintained and removed by a release tube (6) during different phases of system operation, including acclimation and spike preparation. The chamber sits on top of (B) systems controls that are located beneath each chamber and consists of an inflow control valve (1), vortex flow meter (2), temperature sensor (3), pressure sensor (4), servo motor (5), and a rod and piston (6) used to control rapid pressure changes. Note that panel (B) is a drawing to aid distinction of various components.

2. Methods

2.1. System description

Each of the MABL's four chambers measures 45.7 cm inside diameter and 31.2 cm high, giving an interior volume of 51.2 L (Fig. 1(A)). The chambers are made of 3.0-cm thick acrylic glass and sealed at the top and bottom by 5.1-cm thick aluminum. The chamber is surrounded by a plexiglas lens that is flat on all sides. The void between the lens and the chamber is filled with water, allowing non-distorted viewing. The chamber interior is accessed by a removable hatch, which covers a 30.5-cm diameter opening. During operation, the testing area is enclosed by vinyl curtain, providing isolation.

2.2. Water flow and pressure

Water flow through the chambers is controlled by a computer program with graphical user interface (GUI; LabView, National Instruments Corporation, Austin, TX). Flow-through water is delivered to chambers from an elevated head tank (125 L). The GUI adjusts the flow rate (±0.95 L/min of target) allowing the chambers to hold acclimation pressure constant for greater than 72 h. Pressures can be maintained during the acclimation period between ~101 kPa (surface pressure) and ~414 kPa (32 m depth). Because each chamber is controlled separately, the flow and acclimation pressures can be unique to each chamber simultaneously.

2.3. Water quality

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

2.4. Video observation and recording

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

2.5. Experimental procedures

Using the main computer program GUI, each chamber can be operated in four separate operational modes: fill, acclimate, spike preparation, and drain. At any given time, each of the four chambers can be switched in and out of any of the four operational modes without disrupting the other chambers.

The fill mode allows the chamber to be partially filled in preparation for loading fish (or other aquatic organisms) into the chamber. Following hatch closure, latches are securely dogged down (Fig. 1(A)), and the acclimation mode can then be initiated. The acclimation mode delivers water into the chamber at a selected flow rate and acclimation pressure. This mode maintains an "automated air pocket" that allows physostomous species to gulp at the air space and become neutrally buoyant. The acclimation periods are necessary for physoclistous fish to achieve neutral buoyancy or aquatic organisms that do not have a swim bladder so that gases in tissues can equilibrate with surrounding pressures. To accommodate hyper/hypobaric conditions, the air pocket must be completely removed prior to decompression (Fig. 1(A)). The Spike Preparation mode prevents subtle pressure changes by slowly replacing the automated air pocket with water. After the air pocket is removed, a pressure change scenario (referred to in this paper as a spike) is initiated by directing the GUI to load the predetermined pressure simulation profile. This process activates a servo motor that controls a piston (Fig. 1(B)) and subsequently initiates the pressure profile (Fig. 1(A)). The GUI displays the complete exposure instantaneously and saves the actual pressure measurements sampled at 1000 Hz. The drain mode allows air to slowly enter while water is removed from the chamber to a depth of 2.54 cm without disrupting the surface pressure conditions. The chambers may be returned to the acclimation mode if the fish are to be held for an extended post-treatment observation period, at preselected pressure and flow conditions.

3. Case study description

3.1. Fish acquisition and handling

Both hatchery-reared and seaward-migrating juvenile Chinook salmon (*Oncorhynchus tshawytscha*) were exposed to STP treatments. Seaward-migrating yearling and sub-yearling Chinook salmon were collected daily between May 9, 2007, and August 22, 2007, from the Columbia River using the juvenile bypass facility at McNary Dam (*n* = 599). Sampled fish had a mean fork length of 112.8 mm (range, 80–165 mm) and a mean weight of 16.8 g (range, 6–58 g). Fish were held overnight, unfed, in a 125-L circular container with flow-through ambient river water prior to testing. Hatchery-reared yearling and sub-yearling Chinook salmon were either acquired as fry or hatched and reared from eggs at the Pacific Northwest National Laboratory (PNNL) aquatic research laboratory (ARL). These fish were tested at the PNNL ARL between September 10, 2007, and May 30, 2008 (*n* = 862). Sampled fish had a mean fork length of 143.8 mm (range, 82–180 mm) and a mean weight of 34.1 g (range, 6.1–71.5 g). While at this location, the fish were held in 1100-L circular holding tanks with flow-through ambient well water (mean temperature = 16.83 °C ± 0.10 SE) and fed biodiet moist pellets (Bio-Oregon, Longview, WA) ad libitum. The same water was supplied to the MABL during this testing period.

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

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

3.2. Loading fish into the chambers

Each bucket of fish was introduced into a partially filled chamber, and the GUI controls were set to the acclimation mode,

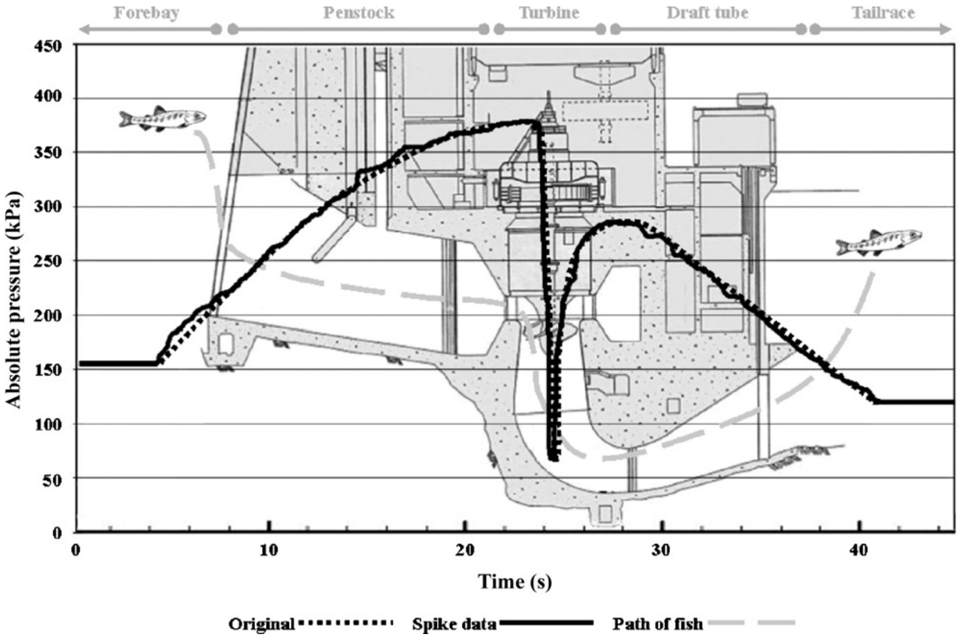

Fig. 2. An example of the simulated turbine passage (STP) pressure profile. Original data (dotted line) were gathered using a sensor fish sent through a Kaplan turbine (Deng et al., 2007) and the spike file measured inside the chamber by the MABL system (solid line). This figure has been modified from Brown et al. (2009). The grey dashed line represents a typical path of a fish (pressure equivalents that the fish would experience are represented by the previously indicated black lines). The lowest pressure a fish is subjected to within the turbine is termed the "nadir" and is illustrated at ~25 s on the *x* axis.

allowing the chamber to fill at a rate of 7.6 L/min and maintain an air pocket. Fish were given at least 16 h to acclimate to pressure equivalents of 7.62 m (175.8 kPa). During this acclimation period, fish were observed via video actively gulping air at the air pocket within the chamber, filling their swim bladders in an attempt to achieve neutral buoyancy. Following the acclimation period, researchers determined the state of buoyancy of each fish, which served as a predictor variable in our analyses.

3.3. *Determination of buoyancy*

Using the video imaging system, each fish was determined to be negatively, positively, or neutrally buoyant. According to Harvey (1963), negatively buoyant fish tend to swim head-up/tail-down to remain off the bottom of the chamber, and they also exhibit elevated tail beat rates. Neutrally buoyant fish in Harvey's study were able to maintain a horizontal position within the chamber

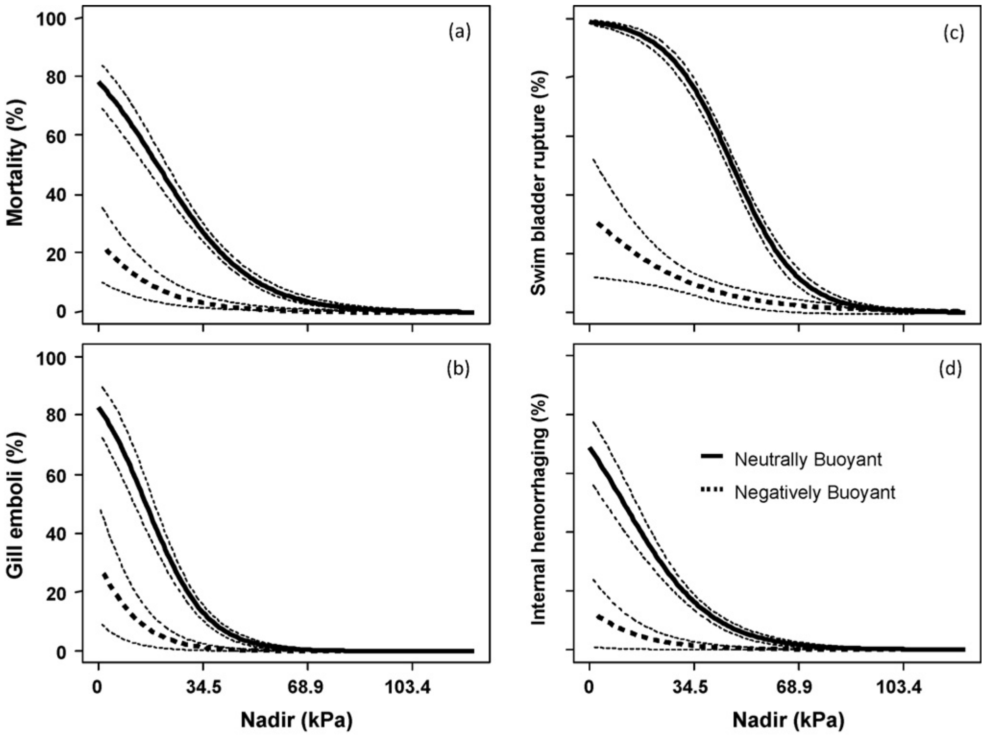

Fig. 3. Resulting percentages of (A) emboli in the gills, (B) swim bladder rupture, (C) internal hemorrhaging (heart, liver, or kidney), and (D) mortality in fish that attained neutral buoyancy after acclimation and fish that did not achieve neutral buoyancy, at a given nadir. Standard deviations are expressed as small hashed lines about the dotted (negative buoyancy) and solid lines (neutral buoyancy).

Table 1

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

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

with minimal fin movement. Symptoms of positive buoyancy were exhibited by fish continuously struggling to move downward in the water column (head-down/tail-up) (Harvey, 1963).

3.4. Simulated turbine passage (STP) exposure

The original pressure data used to derive the pressure profiles applied to STP tests in the current study were collected using an autonomous sensor fish device (Deng et al., 2007). During the exposure, the chamber pressure was increased to approximately 400 kPa over 20 s to simulate fish passing through a hydroelectric turbine intake and approaching the turbine runner (Fig. 2). Brown et al. (2009) suggests that the compression of air filled structures or dissolved gases within tissues are generally not subject to barotrauma. The fish were then subjected to rapid decompression within 0.5–3.5 s to nadir values (the lowest pressure) ranging from 12.1 to 127.9 kPa, simulating passage through the suction side of a runner blade in the runner region. Finally, the program controlled the chamber pressure to simulate movement of fish out of the turbine draft tube and into the tailrace when pressure returned to atmospheric (approximately 101 kPa). The total STP exposure from the pressure increase in the intake to atmospheric pressure in the tailrace lasted approximately 40 s. The actual spike pressure measurements were very close to the targeted pressure profile (Fig. 2).

3.5. Fish removal and necropsy

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

3.6. Statistics

Sequential analysis of deviance based on a logistic link function with a Bernoulli error structure was used to find the independent variables that produced the best fit model for each binary response variable (Kutner et al., 2005). This type of model falls under the umbrella of generalized linear models. Wald chi-square statistics and Akaike's Information Criterion (AIC) values were used to determine best-fit models for each binary response variable. The process of model building began by finding the best single covariate model, based on the Wald chi-square statistics and AIC values, then testing all remaining covariates together with the best single covariate and again testing the model fit. This process continued until no further covariates significantly improved the fit of the model. The presence or absence of a specific injury or mortality served as the binary response variables. Nadir, fish length, and fish weight served as continuous covariates, and state of buoyancy before pressure spike served as a binary covariate. Significance was assessed at $\alpha < 0.05$ and computations were performed using the computing program R (version 2.9.1 The R foundation for statistical computing).

4. Results

As nadir decreased, the incidence of immediate mortality increased, but it was much less common among negatively buoyant fish than neutrally buoyant fish (Fig. 3(A)). Nadir ($P < 0.01$) and buoyancy ($P < 0.01$) were significant predictors of immediate mortality in juvenile Chinook salmon (Table 1). Neither weight ($P = 0.23$) nor length ($P = 0.14$) predicted mortality among the size range of fish we tested. The main model explained 21% of the variability in the model; nadir explained 15%, and buoyancy explained 6%. The non-linear logistic regression equation for the probability of mortality for a given nadir and buoyancy is presented below and the coefficients derived by the model are summarized in Table 2:

$$P_{\text{mortality}} = \frac{e^{-1.132-0.450\times\text{nadir}+2.400\times\text{buoyancy}}}{1+e^{-1.132-0.450\times\text{nadir}+2.400\times\text{buoyancy}}}$$

As nadir decreased, the incidence of emboli in the gills increased. However, gill emboli were observed much less frequently among negatively buoyant fish and were seldom observed unless fish were exposed to nadirs less than approximately 48.3 kPa (Fig. 3(B)). Nadir ($P < 0.01$), buoyancy ($P < 0.01$), and length ($P < 0.01$) were significant predictors of the presence of emboli in the gills of juvenile Chinook salmon (Table 1). Weight was not a significant ($P = 0.61$) variable. The main model explained 33% of the variability in the model; nadir explained 23%, buoyancy explained 6%, and length explained 4%. The non-linear logistic regression equation for the

Table 2

Coefficients for the models as defined by the analysis of deviance. The coefficient estimates divided by their standard error have an asymptotic normal distribution. Coefficients can be used in the response variable specific (mortality, swim bladder rupture, gill emboli, internal hemorrhaging) equation to predict the probability of occurrence at a given buoyancy and nadir following simulated hydro-turbine passage. The lines generated from the equations are expressed in Fig. 3.

Response	Coefficient	Estimate	SE	*Z*	*P*
Mortality	Intercept	−1.13	0.38	−3.01	0.0026
	Nadir	−0.45	0.04	−11.68	<0.0001
	Buoyancy	2.40	0.36	6.69	<0.0001
Gill emboli	Intercept	2.83	0.79	3.59	<0.0003
	Nadir	−0.69	0.06	−10.89	<0.0001
	Buoyancy	2.41	0.44	5.44	<0.0001
	Length	−0.03	0.01	−5.45	<0.0001
Swim bladder rupture	Intercept	−0.70	0.49	−1.44	0.1494
	Nadir	−0.31	0.09	−3.37	0.0008
	Buoyancy	5.03	0.55	9.08	<0.0001
	Nadir.Buoyancy	−0.33	0.10	−3.27	0.0011
Internal hemorrhage	Intercept	−1.85	0.54	−3.42	0.0006
	Nadir	−0.49	0.05	−9.83	<0.0001
	Buoyancy	2.64	0.52	5.07	<0.0001

probability of emboli in the gill for a given nadir, buoyancy, and length is presented below and the coefficients derived by the model are summarized in Table 2:

$$P_{\text{Gill embolism}} = \frac{e^{2.829-0.691\times\text{nadir}+2.413\times\text{buoyancy}-0.028\times\text{length}}}{1+e^{-2.829-0.691\times\text{nadir}+2.413\times\text{buoyancy}-0.028\times\text{length}}}$$

As nadir decreased, the incidence of swim bladder rupture increased and swim bladder rupture occurred much less often among negatively buoyant fish (Fig. 3(C)). Nadir ($P<0.01$), buoyancy ($P<0.01$), and the interaction between nadir and buoyancy ($P<0.01$) were significant predictors of swim bladder rupture in juvenile Chinook salmon (Table 1). The main model explained 38% of the variability in the model. Nadir (22%) and buoyancy (15%) explained most of the variability in the model, while the interaction between nadir and buoyancy explained little (0.4%). The non-linear logistic regression equation for the probability of swim bladder rupture for a given nadir and buoyancy is presented below and the coefficients derived by the model are summarized in Table 2:

$$P_{\text{SB rupture}} = \frac{e^{-0.700-0.309\times\text{nadir}+5.033\times\text{buoyancy}-0.325\times\text{nadir}\times\text{buoyancy}}}{1+e^{-0.700-0.309\times\text{nadir}+5.033\times\text{buoyancy}-0.325\times\text{nadir}\times\text{buoyancy}}}$$

As nadir decreased, the incidence of hemorrhage increased, but it was seen less often in negatively buoyant fish compared to neutrally buoyant fish (Fig. 3(D)). Nadir ($P<0.01$) and buoyancy ($P<0.01$) were significant predictors of hemorrhaging of the liver, heart, or kidney in juvenile Chinook salmon (Table 1). Neither weight ($P=0.09$) nor length ($P=0.13$) predicted mortality among the size range of fish we tested. The main model explained 21% of the variability; nadir explained 15%, and buoyancy explained 6%. The non-linear logistic regression equation for the probability of internal hemorrhage for a given nadir and buoyancy is presented below and the coefficients derived by the model are summarized in Table 2:

$$P_{\text{hemorrhage}} = \frac{e^{-1.849-0.485\times\text{nadir}+2.641\times\text{buoyancy}}}{1+e^{-1.849-0.485\times\text{nadir}+2.641\times\text{buoyancy}}}$$

5. Discussion

5.1. MABL attributes

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

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

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

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

The computer program controls each chamber separately, allowing independent chamber operation. The system also allows remote data collection and delayed data analysis using readily available software products. Together, these attributes make the

MABL an affective barotrauma testing facility that addresses many preexisting limitations of other decompression systems.

5.2. Importance of buoyancy

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

Neutral buoyancy is a delicate balance of opposing forces (i.e., gravity and buoyancy) that minimizes the energy required to maintain a preferred location in the water column (D’Aoust, 1973; Lefrancois et al., 2001). When the mass of the water displaced by a fish equals the fish’s mass, the fish becomes neutrally buoyant. Brown et al. (2009) illustrated that fish acclimated to higher absolute pressures may be at increased risk of mortal injury because they experience a change of pressure of a higher ratio (acclimation pressure vs. exposure pressure), alluding to the importance of an extended acclimation period. Although, the ratio of pressure change is important in predicting injury or mortality (as detailed by Brown et al., 2009), all fish in the current study were acclimated to the same pressure (the pressure present at 7.62 m; 175.8 kPa). Therefore, exposure pressure (nadir) was used as an appropriate predictor of injury and mortality. Combined, this illustrates the need for research examining the maximum depth at which juvenile salmonids of various species can attain neutral buoyancy and a better understanding of this relationship in wild systems.

Many questions surrounding rapid decompression remain, which include delayed mortality and differences in injuries due to taxa-specific morphology and physiology. In this case study using the MABL, juvenile Chinook salmon were tested. Mortality and injuries were more common among neutrally buoyant fish. However, there was less difference between negatively and neutrally buoyant salmon in regard to internal hemorrhaging than immediate mortality and the other injuries we examined. Hemorrhaging of major organs is likely to cause delayed mortality. Furthermore, swim bladder rupture likely makes fish susceptible to delayed mortality through predation since fish will likely not be able to regulate buoyancy. Future research should examine the importance of injuries attained during turbine passage on delayed mortality. Together, our estimates of instantaneous mortality should be considered a conservative representation of total mortality that can occur among turbine-passed juvenile salmonids.

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

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

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

6. Conclusion

This study demonstrates that the MABL is a useful system to test barotrauma because it controls covariates and addresses the limitations in previous barotrauma research. The case study demonstrated the importance of buoyancy, especially for physostomous fish, when assessing barotrauma and resulting immediate mortality. It also indicated the necessity of developing methods that would cause fish to become negatively buoyant prior to turbine passage, potentially reducing physiological injury and mortality in these economically and ecologically important fish species.

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