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# A Preliminary Assessment of Barotrauma Injuries and Acclimation Studies for Three Fish Species

## Final Report

February 2016

RS Brown
RW Walker
JR Stephenson

U.S. DEPARTMENT OF ENERGY

Prepared for Manitoba Hydro
under an Interagency Agreement with the
U.S. Department of Energy under
Contract DE-AC05-76RL01830

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Pacific Northwest National Laboratory
Richland, Washington 99352

# Summary

Fish that pass hydro structures either through turbines, deep spill, or other deep pathways can experience rapid decreases in pressure that can result in barotrauma. In addition to the morphology and physiology of the fish's swim bladder, the severity of barotrauma is directly related to the volume of undissolved gas in fish prior to rapid decompression and the lowest pressure the fish experience as they pass through hydro structures (termed the "nadir"). The volume of undissolved gas in fish is influenced by the depth of acclimation (the pressure at which the fish is neutrally buoyant); therefore, determining the depth at which fish are neutrally buoyant is a critical precursor to determining the relationship between pressure changes and injury or mortality.

White Sturgeon (*Acipenser transmontanus*), Walleye (*Sander vitreus*), and Tiger Muskie (*Esox lucius* X E. *masquinongy*), were studied to better understand the likelihood of barotrauma when they pass through hydro structures. The pressure at which Walleye and Tiger Muskie were able to become neutrally buoyant was easily measured in pressure chambers as most fish hovered within the water column after being held overnight at a consistent acclimation pressure. Thus, after determining acclimation depth data, the research for these two species focused on understanding how they were influenced by rapid decompression along a range of pressure changes. This was done to determine how many individual Walleye and Tiger Muskie would be required to develop a robust criteria relationship between rapid pressure change and injury and mortality.

After analysis of the relationships between pressure change and barotrauma were conducted on a limited scale, a power analysis was conducted. This analysis determined that it would be necessary to test a total of 300 Walleye and 300 Tiger Muskie (or Northern Pike) to derive a robust relationship between pressure changes and barotrauma. This could be used to provide guidelines for fish passage with minimal injury or mortality. These fish would need to be rapidly decompressed and evenly spaced across a range of ratios of pressure change. Testing of a limited number of Northern Pike should also be conducted to validate that the use of Tiger Muskie is an appropriate surrogate for Northern Pike.

Determining the depth of acclimation of White Sturgeon was not as simple as doing so for Walleye and Tiger Muskie, and the majority of White Sturgeon research for this project was done to help understand at what depths, if any, they would become neutrally buoyant. When examined in the hyper/hypobaric chambers, they did not become neutrally buoyant near surface pressure (~101 kPa absolute), and few of the Sturgeon became neutrally buoyant at higher pressures, reflecting fish being deeper in the water column than surface pressure (>101 kPa; with ~202 kPa equating to 10 m of water depth). This lack of acclimation was observed even when the Sturgeon were held at these pressures for a relatively long period of time (up to 5 days). Therefore, it is critical to develop methods for determining how Sturgeon regulate their buoyancy, what physiological mechanisms are involved, and their boundaries or thresholds. This information is vital to gaining an understanding of how pressure-related injuries may affect Sturgeon being entrained in turbines and spillway structures. This pilot-scale effort did not provide suitable information about the point at which the Sturgeon could become neutrally buoyant; this makes it impossible to derive enough information to determine the relationship between pressure changes and Sturgeon injury. Further research efforts are suggested which could advance the understanding of the vulnerability of Sturgeon, Walleye, and Northern Pike to barotrauma.

# Acknowledgments

Funding for the research described in this report was provided by Manitoba Hydro. The authors thank Manitoba Hydro staff, including Stephanie Backhouse, Cheryl Klassen, and Marilynn Kullman. We would like to thank Mike Erickson and Steve Mansfield of the Washington State Department of Fish and Wildlife. We would also like to thank Shay Wolvert and Holly McLellan of the Confederated Tribes of the Colville Reservation's Resident Fish Division. We extend appreciation to John Skalski and Rich Townsend, University of Washington, for their technical insights and input. We also thank the many staff at the Pacific Northwest National Laboratory whose diverse professional expertise contributed to the success of this study: David Geist, Stephanie Liss, Alison Colotelo, Jill Janak, Neil Ashton, Amy Goldman, and Tim Linley.

The Pacific Northwest National Laboratory animal facilities used in this research are accredited by AAALAC International, the Association for Assessment and Accreditation of Laboratory Animal Care. Fish were handled in accordance with federal guidelines for the care and use of laboratory animals, and protocols for our study were approved by the Institutional Animal Care and Use Committee at Battelle–Pacific Northwest Division. The Pacific Northwest National Laboratory is operated by Battelle for the U.S. Department of Energy under Contract DE-AC05-76RL01830.

# Acronyms and Abbreviations

| | |
|---|---|
| ARL | Aquatics Research Laboratory |
| CT | computed tomography |
| d | day(s) |
| g | gram(s) |
| kPa | kilopascals |
| LRP | log of the ratio of pressure change |
| m | meter(s) |
| MABL | mobile aquatic barotrauma laboratory |
| min | minute(s) |
| mm | millimeter(s) |
| PSI | Pounds for square inch |
| PSIA | Pounds for square inch absolute |
| PNNL | Pacific Northwest National Laboratory |
| RPC | ratio of pressure change |
| STP | simulated turbine passage |
| WDFW | Washington Department of Fish and Wildlife |

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# 1.0 Introduction

When moving downstream through hydro structures, fish pass via structures such as spillways, turbines, bypass facilities, or weir gates, all of which can expose fish to varying degrees of pressure changes (Trumbo et al. 2013). Turbine passage is typically considered the most harmful fish passage route due to the possibility of encountering injuries from blade strike and shear forces in addition to being exposed to pressure changes (Deng et al. 2005, 2007, 2010; Čada et al. 2006; Baumgartner et al. 2014; Brown et al. 2009). Turbine design and size (type, diameter, and operational speed) also influence injury during turbine passage (Brown et al. 2012a). Blade strike susceptibility is largely proportional to body size (Franke et al. 1997) and while not all fish are exposed to damaging levels of shear when passing turbines (Deng et al. 2007), they are exposed to rapid pressure changes (Deng et al. 2010).

When fish are exposed to rapid pressure changes, they are vulnerable to barotrauma, (injury associated with changes in barometric pressure; Brown et al. 2009). Barotrauma can include emboli in the gills, hemorrhaging, ruptured swim bladder, and exopthalmia (eye bulging out of the eye socket; see Figure 1 for examples). Also, damage may occur when the swim bladder over-inflates and crushes internal organs. Brown et al. (2012d) found that barotrauma in juvenile salmonids passing hydroturbines was largely due to the expansion of undissolved gas within the swim bladder, the rupture of the swim bladder, and damage caused when escaping gas caused hemorrhaging, emboli, and exopthalmia. Therefore, the presence, form, function, and type of swim bladder, whether it is inflated or not, and the amount of gas within it can influence the vulnerability of fish to barotrauma.

Swim bladders can either be open or closed or not present in fish (Colotelo et al. 2012; see figure in Appendix C), and this factor can greatly influence susceptibility to barotrauma. Fish with open swim bladders (termed physostomes), such as Salmon (*Oncorhynchus* spp.), Sturgeon (*Acipenseridae*) and Pike (*Esocidae*), have a connection between the esophagus and the swim bladder, meaning fish can regulate swim bladder volume and buoyancy, by either gulping air at the water surface or expelling gas. This connection allows them to evacuate gas from the swim bladder and expel it from the mouth and gill covers during rapid decompression, which can make them less vulnerable to barotrauma than fish with a closed swim bladder (termed physoclists; Deng et al. 2012). Physoclistous fish, such as Walleye (*Sander vitreus*) and Burbot (*Lota lota*), regulate the volume of gas in the swim bladder by exchanging gas from the blood through an organ known as the rete mirabile (for examples see Figure 2). This exchange process can be considerably slower than gas exchange via the pneumatic duct, and as such, physoclistous fish can be more vulnerable to barotrauma. For example, Bluegill (*Lepomis macrochirus*), which are physoclists, have been shown to have greater injury and mortality than physostomous juvenile Chinook Salmon (*Oncorhynchus tshawytscha*), when exposed to similar rapid decompression scenarios (Abernethy et al. 2001). Variability in injury can also be due to the high variability in expulsion of gas past the pneumatic duct (a sphincter or cylindrical muscle that regulates gas entering or leaving the swim bladder) during decompression. This can vary both within and among species (Brown et al. 2014).

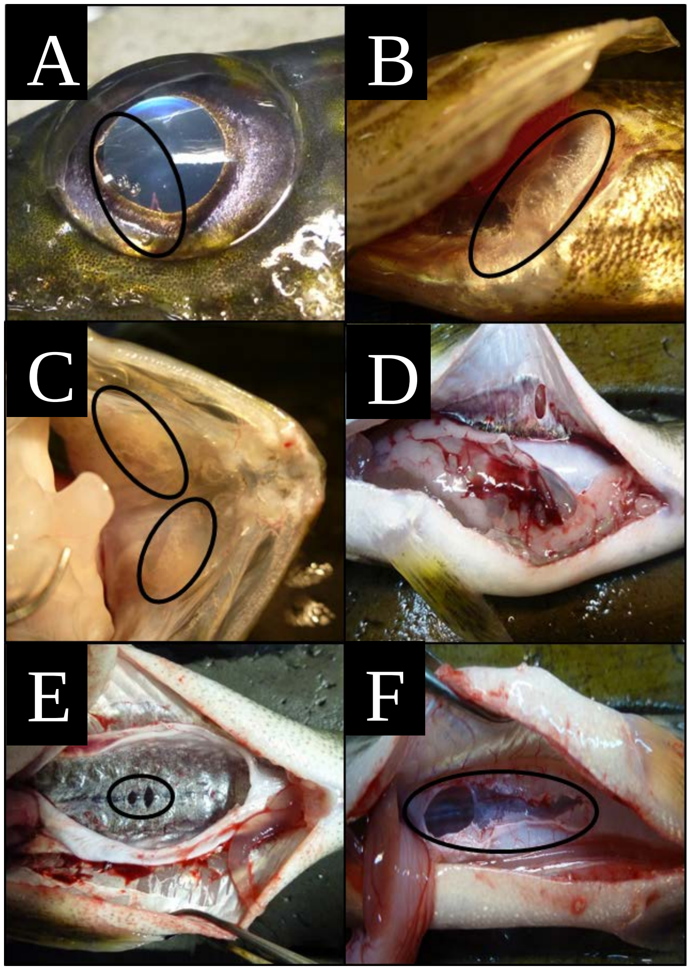


**Figure 1**. Examples of types of injuries noted among juvenile Walleye and Tiger Muskie exposed to simulated turbine passage within a range of ratio of pressure changes (RPCs) of hydro passage (from 3.8 to 20.0). Injuries can include emboli and hemorrhaging in the eye (A), gas in the pericardial window (B), emboli in the head cavity behind the eyes (C) hemorrhaging along the swim bladder from the kidneys and hemorrhaging from the liver and spleen (D), swim bladder ruptures located along the kidney on the dorsal side of the swim bladder (E) and the rupture of a juvenile Tiger Muskie swim bladder (F).

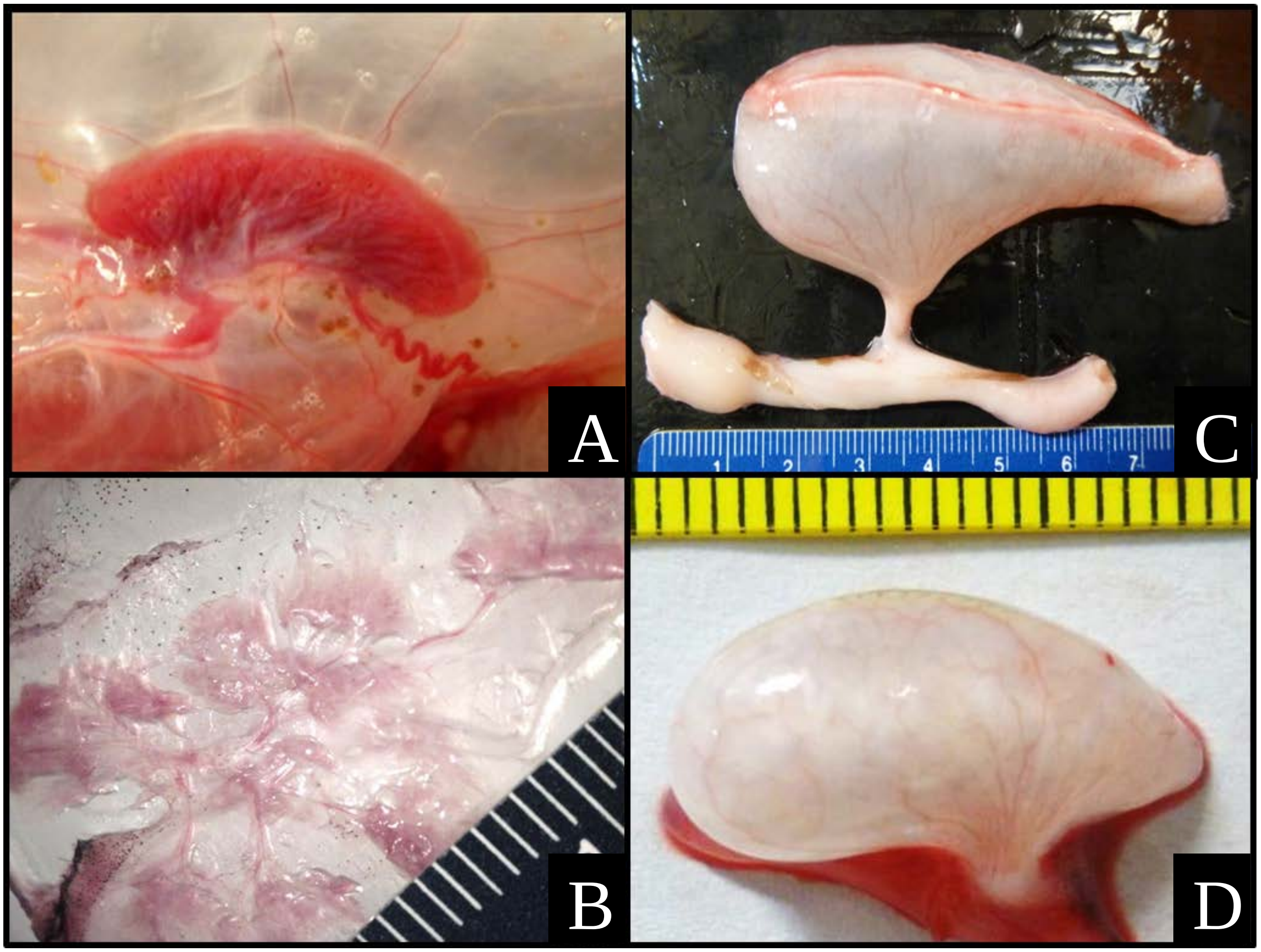


**Figure 2**. Examples of the rete (bed of vasculature) located on the swim bladder of a Speckled Longfin Eel (*Anguilla reinhardtii*; A) a Walleye (B; the darker tissue), a White Sturgeon, and Lake Sturgeon. The rete of the Eel is shown to indicate the very dense bed of vasculature that can be present on swim bladders. The swim bladder of a White Sturgeon (C) and Lake Sturgeon (D) are shown for comparison and contrast because there is little vascularization visible on their swim bladders. (Photo credit for panel A: Brett Pflugrath, New South Wales Department of Primary Industries, Australia.)

Because fish injury following rapid decompression is predominantly associated with expansion of pre-existing gases (Brown et al. 2012d), the prediction of barotraumas in fish passing through hydro structures requires a firm understanding of the degree to which gas expands within fish when they are decompressed. Based upon Boyle's law, one of the primary determinants of swim bladder volume change (and therefore likelihood of injury) is the ratio of pressure change (RPC) experienced by the fish during passage. This ratio may be as simple as dividing the pressure associated with the depth to which fish are acclimated and neutrally buoyant prior to passage, by the nadir (lowest pressure) experienced during infrastructure passage.

The following analogy serves to illustrate the importance of the ratio of pressure change, rather than absolute pressure change, to swim bladder volume and thus the potential for barotrauma. If a fish is brought to the surface (101 kPa) from an acclimation depth of 10 m (202 kPa) at which it is neutrally buoyant, it will experience a ratio of pressure change of 2 (202 kPa/101 kPa = 2), which implies that swim bladder volume would double (in the absence of body wall constraints). In this scenario, the absolute pressure change is 101 kPa (202 kPa - 101 kPa = 101). This doubling of swim bladder volume would also occur in a fish acclimated to the surface of the water column (101 kPa) that passes through a hydroturbine with a nadir pressure of 50.5 kPa because the ratio of pressure change is again 2 (101 kPa/50.5kPa = 2), even though the absolute pressure change is only 50.5 kPa—half the value of the example above. Understanding the significance of Boyle's law and its potential impacts on fish can inform the hydraulic design of hydroturbines and other water control structures to control the nadir pressure and minimize the ratio of pressure change.

Understanding the factors involved in the RPC to which a given species may be exposed is crucial. First, the depth (or equivalent pressure) at which the species can acclimate and become neutrally buoyant should be identified, because it will influence the maximum RPC that a fish may experience when passing through hydro structures. Second, how fish acclimated to depth respond to rapid decompression should be investigated. Using hyper/hypobaric chambers, test fish can be acclimated to depths where fish are neutrally buoyant in the wild, and exposed to a range of nadir pressures in order to attain a range of RPCs. Exposing fish to a large range of RPCs enables researchers to identify a threshold above which fish are more likely to be injured or die as a result of rapid decompression.

The purpose of this research was to examine the effect of pressure change on survival and injury rates of Walleye, Lake Sturgeon and Northern Pike, by conducting pilot-scale testing and power analysis to determine sample sizes required to develop a robust relationship between pressure change (simulating passed through hydro structures) and injury/mortality. Due to limitations in species availability, Tiger Muskie (*Esox masquinongy X Esox lucius*) were used as a surrogate for Northern Pike and White Sturgeon (*Acipenser transmontanus*) were used as a surrogate for Lake Sturgeon. Little work has been done to establish the effect of pressure changes simulating turbine passage on the study species, however, as Walleye are physoclists, much less variability in injury is expected. This pilot-scale effort is aimed at understanding the relationship between pressure changes and fish injuries well enough so that larger scale research can be planned and conducted to more fully understand these relationships.

# 2.0 Methods

## 2.1 Fish Sources and Holding Conditions

Juvenile White Sturgeon were obtained in November 2013 and November 2014 from the Washington Department of Fish and Wildlife (WDFW) Columbia Basin Hatchery (Moses Lake, WA). Juvenile Tiger Muskies approximately 2 years old) were obtained in June 2014 from the WDFW Ringold Hatchery (Mesa, WA). Juvenile Walleye were obtained in August 2013 and June 2014 from the Confederated Tribes of the Colville Reservation (Nespelem, WA). All fish were held and reared at the Aquatic Research Laboratory (ARL) at Pacific Northwest National Laboratory (PNNL; Richland, WA). Juvenile Sturgeon and Tiger Muskie were held in 600 L circular tanks and Walleye were held in a 1200 L circular tank. All tanks were supplied ambient temperature flow-through Columbia River water, passed through a drum sand filter and ultraviolet light water purification. The photoperiod in the ARL was set to replicate the natural outdoor photoperiod for Richland, Washington, using overhead fluorescent lighting. Juvenile Sturgeon were fed an ad libitum ration of Bio-Oregon (Longview, WA) pellets while juvenile Tiger Muskie and Walleye were fed live Chinook Salmon weekly. The fish selected for tests were not fed 24 h before testing.

## 2.2 Mobile Aquatic Barotrauma Laboratory

The barotrauma and acclimation studies were assessed using the Mobile Aquatic Barotrauma Laboratory (MABL) located at the ARL. The system consists of four hyper/hypobaric chambers. Stephenson et al. (2010) describe in detail the physical characteristics, capabilities, and operation of the MABL.

The exposure to rapid decompression was done after acclimating fish for 16 to 24 h in the chambers at 146.2 kPa (equivalent to 4.6 m of water depth). This acclimation period allowed fish to become neutrally buoyant as indicated by their posture in the water column and fin movements; typically they hovered off the bottom of the chambers with very little fin movement, similar to images illustrating this by Pflugrath et al. (2012). Exposure to rapid decompression (see methods Stephenson et al. 2010) simulating turbine passage (STP) through a Kaplan style turbine was done at a different range of nadir values, rate of pressure change and RPC for each species.

Within 15 minutes after exposing fish to STP, fish were euthanized with an overdose of MS-222 (tricaine methanesulfonate at a concentration of 250 ppm). Fish were then measured for length and weight and necropsied to determine if any barotrauma injuries (see Brown et al. 2012b) were present and injuries, such as emboli and hemorrhaging in the muscle tissue, eyes, fins, lateral line, and internal organs as well as exopthalmia, inverted stomachs, ruptured vasculature and ruptured swim bladder, were recorded (see Figure 2). Research completed with juvenile Chinook Salmon found that certain injuries (i.e., swim bladder rupture, emboli in the gills and pelvic fins, hemorrhaging of the liver, heart and kidney) were highly associated with immediate mortality as described by Brown et al. (2012b). Fish that had these injuries were recorded as mortally injured, the same as fish that died immediately following STP. However, injuries associated with mortality for Chinook Salmon might not lead to mortality in other species such as Tiger Muskie, Walleye, or White Sturgeon, and our estimate of mortality might be conservative.

## 2.3 Tiger Muskie Simulated Turbine Passage Testing

Fifty juvenile Tiger Muskie (a physostome with an open swim bladder [Figure 2]; used as a surrogate for Northern Pike) were examined. They had a mean fork length (FL) of 187 mm (range 122–259 mm FL) and mean weight of 41.1 g (range 8.2–103.6 g). They were exposed to rapid decompression (see methods Stephenson et al. 2010) simulating turbine passage through a Kaplan style turbine. The nadir was a mean of 40.7 kPa (range 13.1–73.8 kPa) and the RPC was a mean of 979 kPa/s (range 538–1572 kPa/s). The ratio of pressure change (nadir pressure/acclimation pressure) was a mean of 4.2 (range 1.7–11.2).

## 2.4 Walleye Simulated Turbine Passage Testing

The 56 juvenile Walleye (a physoclist with a closed swim bladder) tested had a mean FL of 237 mm (range 178–319 mm) and a mean weight of 117.0 g (range 40.9–272.4 g). Exposure to rapid decompression simulating turbine passage through a Kaplan style turbine was done at a mean nadir of 38.6 kPa (range 6.9–73.1 kPa) and the rate of pressure change was a mean of 1034 kPa/s (range 565–2193 kPa/s). The RPC was a mean of 4.3 (range 1.6–20.0). Walleye did not always become neutrally buoyant during the 16 to 24 h acclimation period, therefore some individuals had to be slowly decompressed until they started to float off the bottom of the chamber in order to determine the pressure at which they were neutrally buoyant prior to STP testing.

## 2.5 Statistical Analysis for Simulated Turbine Passage Testing of Tiger Muskie and Walleye

The pressure at which a fish is neutrally buoyant and the nadir pressure were used to calculate the RPC for each fish exposed to STP. The RPC was then log transformed (also referred to as the log ratio pressure [LRP]) and used to determine an appropriate sample size to attain a robust probability of mortality curve over a range of LRPs for each fish species. Detailed statistical methods are outlined in Appendix A.

## 2.6 Ability of Sturgeon to Attain Neutral Buoyancy and Rete Activity

Juvenile White Sturgeon tested had a mean FL of 317 mm (range 242–400 mm) and a mean weight of 193.0 g (range 82.3–417.5 g). White Sturgeon in this assessment did not behave similarly to Tiger Muskie or Walleye during the acclimation phase of STP; they did not exhibit behavior that provided an obvious state of buoyancy. To effectively examine injuries as a result of barotrauma in Sturgeon, it is important to know the state of buoyancy of the fish. White Sturgeon were loaded into a hyper/hypobaric chamber and acclimated to 117.2 kPa or 146.2 kPa, which equates to 1.5 m and 5 m of depth in the water column, respectively. To aid in understanding the buoyancy regulation of the Sturgeon, they were held in the chambers under these pressures from 1 to 5 d.

Because Sturgeon were always observed to appear negatively buoyant at the pressures they were held under, a test was devised to determine if the fish fill their swim bladder by gulping air at the water surface or if there may be some latent rete activity. To facilitate this test, 24 fish were held with access to the water surface and 13 other fish were held with restricted access to the water surface by placing a screen in the chamber just below the water surface. After the initial acclimation period, fish were decompressed slowly, over a mean of 4 min (range 1–8 min), to determine when the fish was neutrally buoyant, or when the fish expelled gas, whichever happened first.

Fish with access to the water surface and fish with restricted access to the water surface were compared to determine their rete activity and ability to become neutrally buoyant. This was done by plotting the difference in the acclimation pressure and the pressure at which a fish was neutrally buoyant (or expelled gas) during slow decompression, whichever happened first.

It is possible that hatchery-reared fish that have never been in the wild may not be representative of wild fish. It could be that hatchery-reared fish do not inflate their swim bladders because they are never in deep-water habitats. To gain insight into the variability of swim bladder morphology among White Sturgeon, fish were necropsied after buoyancy regulation experiments and observations of swim bladder size and state of inflation were recorded.

## 2.7 White Sturgeon Simulated Turbine Passage Testing

Because the Sturgeon seldom appeared to add gas to their swim bladder and become neutrally buoyant during their acclimation period, testing White Sturgeon using methods described for Walleye and Tiger Muskie was not possible. However, to gain some possible insight into the susceptibility of White Sturgeon to barotrauma, 46 fish were placed in hyper/hypobaric chambers at an acclimation pressure of 146.2 kPa for 16 to 24 h prior to STP exposures. The injuries observed could provide guidance for determining the pathways of injuries and the type of injuries that could be expected in follow-up research, but should not be viewed as a relationship between the injuries noted and the nadir pressures to which Sturgeon were exposed. All of these fish were negatively buoyant prior to STP exposure. Because buoyancy prior to STP exposure could not be determined, the RPC could not be calculated. Evaluated juvenile White Sturgeon had a mean FL of 260 mm (range 145–338 mm) and a mean weight of 120.1 g (range 17.4–236.4 g). The nadir was a mean of 4.0 kPa (range 1.8–10.3 kPa) and the rate of pressure change was a mean of 273 kPa/s (range 130–396 kPa/s). White Sturgeon were euthanized and necropsied following the same methods described above. Observations of the swim bladder were made (e.g., state of inflation and length) during the necropsy.

# 3.0 Results

## 3.1 Ability of Walleye and Tiger Muskie to Attain Neutral Buoyancy

The information about the injury and mortality presented above was collected after the fish were allowed 16 to 24 h to "acclimate" or become neutrally buoyant in the pressure chambers set to the depth equivalent of being 4.6 m deep in the water column (146 kPa). Although many fish became neutrally buoyant near the pressure within the chambers, there was a wide range of pressures that both Walleye and Muskies were found to be neutrally buoyant. This was determined by jogging the piston that is part of the chamber system, up and down until the fish were neutrally buoyant. Then this information could be noted and after rapid decompression was conducted, the RPC could be determined.

Only 1 of the 50 Walleyes (2%) was neutrally buoyant at exactly the pressure where they were held in the chambers. The Walleyes that were not neutrally buoyant at the pressures in the chambers were neutrally buoyant at a mean pressure of 123 kPa (range 97–137 kPa), the depth equivalent of 2.1 m (range ~surface pressure – 3.7 m). Most (62%) of the Tiger Muskies became neutrally buoyant after being held 16 to 24 h in the chambers. The Muskies that did not become neutrally buoyant were neutrally buoyant at a mean of 122 kPa (range 98 – 143 kPa), the depth equivalent of 2.1 m of depth in the water column (range ~surface pressure – 4.3 m).

**Table 1**. List of injuries for Walleye (N = 56) and Tiger Muskies (N = 50) for fish exposed to rapid decompression simulating turbine passage. The number of fish with injuries is also shown for each species and some fish had multiple injuries; 36% for Walleye and 12% for Tiger Muskie.

| | | Species[(a)] | |
|---|---|---|---|
| Injuries | Location | Walleye | Tiger Muskie |
| Swim bladder rupture* | | 11 (19.6) | 11 (22.0) |
| Emphysema | | | |
| | Pericardial window | 17 (30.4) | 1 (2.0) |
| | Gill[(b)] | 3 (5.4) | 0 |
| | Eye | 1 (1.8) | 0 |
| | Fin | 1 (1.8) | 0 |
| | Renal | 5 (8.9) | 0 |
| | Pericardial | 1 (1.8) | 0 |
| | Head sinus | 3 (5.4) | 0 |
| Hemorrhaging | | | |
| | Eye | 9 (16.1) | 0 |
| | Fin | 2 (3.6) | 8 (16.0) |
| | Capillaries | 7 (12.6) | 0 |
| | Renal[(b)] | 9 (16.1) | 0 |
| | Hepatic[(b)] | 3 (5.4) | 1 |
| | Pericardial[(b)] | 5 (8.9) | 0 |

(a) Values within parentheses are the percent of fish with injuries.
(b) Injuries that are highly associated with mortal injury in Chinook Salmon. Emphysema in most fins is not associated with mortality, except when found in the pelvic fin.

## 3.2 Walleye and Tiger Muskie Rapid Decompression Testing

For Tiger Muskie (N = 50) and Walleye (N = 56), statistical analysis determined that it would be necessary to use a sample size of 300 fish for each species to attain a robust relationship between rapid pressure change and fish injury and mortality (see Appendix A for statistical methods and results). These exposures would have to be evenly distributed across a range of RPCs to develop a robust criteria relationship between rapid pressure change and mortality and injury (Appendix A).

Walleye had more injuries in most categories than Tiger Muskie (Table 1). Among both Walleye and Muskie, there were no immediate mortalities (within 15 minutes of the rapid pressure decrease). It was noted that Walleye swim bladder rupture was often not very noticeable. The rupture was seen at the upper part of the swim bladder and the rupture could be overlooked unless care was taken to examine the swim bladder thoroughly. It was noted among Walleye that the swim bladder could rupture and the escaping gas was noted to travel forward into the head sinuses. This is likely why there was relatively higher damage to the eyes of the Walleye. This was not noted among the other two species.

## 3.3 Ability of Sturgeon to Attain Neutral Buoyancy and Rete Activity

Of the 37 White Sturgeon that were slowly decompressed, all were negatively buoyant at or near surface pressure. Only 9 (24.3%) of the Sturgeon floated (when slowly exposed to pressures between surface and vapor pressure; 0 kPa) and were observed to become positively buoyant. Positive buoyancy was indicated by the fish either floating or actively swimming toward the bottom of the chamber where it is thought these fish were more at rest. Twenty-one (56.8%) of the 37 fish expelled gas without floating, and 7 (18.9%) fish did not float or expel gas.

Sturgeon with access to the water surface expelled gas or became neutrally buoyant at a mean of 9.5 kPa (range 2.5–16.1 kPa; see Figure 3). Sturgeon without access to the water surface expelled gas or became neutrally buoyant at a mean of 10.2 kPa (range 7.1–13.4 kPa). Thus it appears that the rete did not play a role in filling the swim bladder.

To gain insight into the variability of swim bladder morphology among White Sturgeon, fish were necropsied after buoyancy regulation experiments and observations of swim bladder size and state of inflation were recorded. Fish that floated during buoyancy testing in the pressure chambers had inflated swim bladders of relatively large sizes (>35 mm in length) compared to some of their cohort, as did the fish that expelled gas near the pressure level in the pressure chambers. However, many fish had relatively small swim bladders (<25 mm in length) or large swim bladders (>35 mm) that were nearly deflated, or completely deflated (Figure 4) among fish of similar sizes (range 242–324 mm FL).

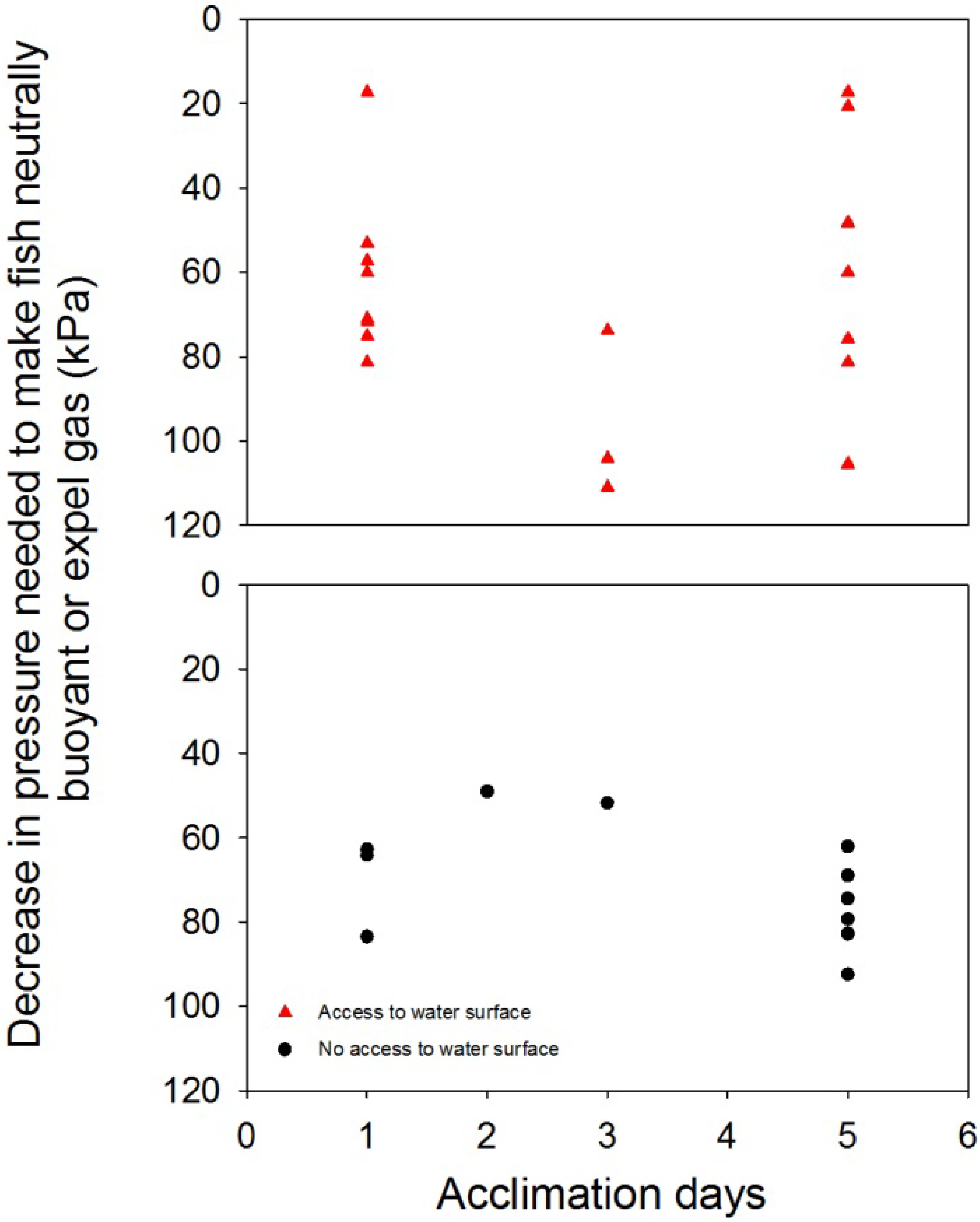


**Figure 3**. White Sturgeon with (red triangle) and without (black circle) access to the water surface gas were acclimated to either 117.2 kPa or 146.2 kPa in hyper/hypobaric chambers over 1 to 5 d, and then decompressed slowly (range 1–8 min) until they expelled gas or moved toward neutral buoyancy. The upper panel (red triangles) illustrates the fish with access to the water surface and the lower panel (black circles) shows fish that did not have access to the water surface. These data points represent individual fish and the acclimation pressure minus the pressure where fish were neutrally buoyant (or expelled gas).

## 3.4 White Sturgeon Simulated Turbine Passage Testing

Among the 46 fish exposed to STP without determination of buoyancy prior to exposure, 16 fish (34.8%) were injured. The injured fish had a mean FL of 225 mm (range 145–319 mm) and a mean weight of 75.3 g (range 17.4–199 g). These fish were exposed to very low nadirs; a mean of 2.8 kPa (range 2.1–4.1 kPa) and the rate of pressure change was a mean of 298 kPa/s (range 234–390 kPa/s).

The majority of the injuries included embolism in the head sinuses (noted in 9 fish; 19.6%) and pericardial (heart) window (noted in 8 fish; 17.4%). There was only one (2.2%) case of mild gill embolism, emphysema in the pectoral fins, hemorrhaging in the eyes, and bruising or hematomas in the swim bladder (e.g., four separate fish experienced those injuries). Among Sturgeon, there were no immediate mortalities (within 15 minutes of the rapid pressure decrease).

Among the 46 fish exposed to STP, 10 (21.7%) of them displayed active movements after the exposure. Six (13.0%) of the fish were agitated after the pressure exposure while five (10.9%) were swimming in the chamber. One fish (2.2%) was twitching after the exposure and one (2.2%) was trying to swim downward. Eight (17.4%) of the fish that had these behaviors were exposed to low nadirs, ranging from 2.4 to 3.4 kPa. Only two (4.3%) of the fish were exposed to a higher nadir of 7.2 kPa.

# 4.0 Discussion

Determining the relationship between pressure change and injury among Muskie and Walleye was not difficult because the fish became neutrally buoyant fairly quickly and their behavior was indicative of being neutrally buoyant (Pflugrath et al. 2012). This made determining the sample sizes for future barotrauma research possible (N = 300; see Appendix A). However, the study type may influence the number of fish that should be used to have a statistically robust study.

When a study includes a wide variety of sizes, more research may be needed to determine if there are differences in barotrauma among fish sizes. For both Walleye and Tiger Muskie additional rapid decompression testing may be needed if there is a large difference in barotrauma vulnerability among fish sizes. There may be minor differences in some fish species. Or, there may be larger differences among fish sizes as Shrimpton et al. (1990) noted. They determined that smaller Rainbow Trout had a higher gas pressure release threshold of the pneumatic duct than larger fish. Thus, the larger fish may be more able to expel gas when decompressed. Because Tiger Muskies and White Sturgeon were used as surrogate species for Northern Pike and Lake Sturgeon, future testing should also be conducted using the species of concern to validate their use as surrogates.

Although the mean exposures were very similar for Muskie and Walleye (mean RPC = 4.2 for Muskie and 4.3 for Walleye), they were exposed to a wide range (1.6–20.0) of ratios of pressure change. The exposures at the upper end of the RPC for each species were very high (11.2 for Muskie and 20.0 for Walleye). These ratios would indicate that the undissolved gas in the swim bladder or other areas (like the digestive tract or a rete in the eye) would increase by 11.2 and 20.0 times when exposed to rapid decompression. These exposures may be uncommon or do not realistically occur during turbine passage. However, to get an accurate idea of the potential for mortality or injury of fish to barotrauma, it is critical for fish to be tested over a wide range of RPCs so that the range encompasses the threshold where injury or mortality increases and then plateaus. Studies could identify these passage conditions by using a Sensor Fish, an autonomous sensor package, consisting of three-dimensional (3D) rotation sensors (i.e., three-axis gyroscope), 3D linear acceleration sensors (i.e., three-axis accelerometers), a pressure sensor, a temperature sensor, and a 3D orientation sensor (i.e., three-axis magnetometer; Deng et al. 2014, 2015).

Data collected by Sensor Fish can be paired with laboratory data to understand the susceptibility of fish to barotrauma. However, it is important to understand the depth at which fish are realistically neutrally buoyant to create a robust relationship that appropriately pairs Sensor Fish data with laboratory research. When Sturgeon were examined in the pressure chambers, none of them appeared to become neutrally buoyant at near surface pressure or the pressure present at the 5 m depth. The lack of neutral buoyancy was unexpected because it is common for both biologists and anglers to see bubbles coming from Sturgeon as they are brought to the surface, which suggests that the fish are neutrally buoyant at depths well below surface pressure. However, it is also possible that the fish are expelling gas and then diving as a way to aid in evading a predator; Harvey et al. (1968) called this a "sounding response," which is common among some species of juvenile salmonids. However, biologists researching Sturgeon have noted that smaller Sturgeon do not tend to make the gas expulsions that larger Sturgeon do (Personal correspondence, Phil Bates, Idaho Power Company). In addition, it is unlikely that large sturgeon would be provoked to take actions that would be considered to be avoiding predation because in most of their range there are predators that could influence the fish.

Because knowing a realistic acclimation pressure is critical to understanding barotrauma, several techniques were employed to further the understanding of the state of the Sturgeon before passing hydro structures. One factor examined was the amount of time Sturgeon stay at pressures higher than surface pressure (reflecting deeper water). It was initially hypothesized that over a short period of time (1 - 2 d), Sturgeon might become accustomed to the chambers and use the bubble at the surface to add gas to their swim bladders. It was also theorized that it may be possible for Sturgeon to use the vasculature on their swim bladders to add gas to their swim bladders. It now appears unlikely that this is the case because the sizes and species of Sturgeon we examined had little vasculature on their swim bladders. This was the case for both White and Lake Sturgeon (see Figure 2). However, other fish species that are physostomes have an active rete (see Figure 2 for an example of the vascular rete in an Eel, which is a physostome). The Sturgeon did not become neutrally buoyant after being held for up to 5 d either with or without access to a bubble at the surface of the chambers.

Because none of the White Sturgeon became neutrally buoyant at acclimation pressures, techniques to quantify the volume of gas in them are necessary to ensure accurate characterization of barotrauma injuries. Because the probability of mortality or injury due to barotrauma is dependent on the amount of gas in the swim bladder of fish, Sturgeon could be less susceptible to barotrauma if they only partially inflate their swim bladders under field conditions. However, more research needs to be done to quantify the volume of undissolved gas Sturgeon have in their bodies prior to turbine or deep spill passage.

Another thing to consider when determining the vulnerability of Sturgeon to barotrauma is whether the fish size or other characteristics (i.e., swim bladder morphology, Figure 4) of the stocked hatchery-reared fish accurately reflect fish in the wild, or if their physiology possibly changes after stocking into the wild (for juvenile stocking programs) or when they are held in deep test facilities in laboratories. The inflation levels of the swim bladders of necropsied fish varied greatly among individual White Sturgeon (Figure 4), but it is unclear whether these differences reflect hatchery-related effects on fish, and whether they are representative of wild fish in regard to swim bladder function. The primarily benthic life history of Sturgeon may also have complicated this research. It is not known to what extent Sturgeon or other benthically oriented species can or need to become neutrally buoyant by gulping air into their swim bladders.

The buoyancy state of Sturgeon may also vary with life stage. For example, the swim bladders of small juvenile American Eels (*Anguilla rostrata*) have very little vasculature to modify their buoyancy as juveniles, but by the time adults are making downstream migrations back to the ocean, they have very active retes and are very efficient at regulating their buoyancy. Also, differences in the amount that salmonids (Shrimpton et al. 1990; Greif et al. 2013) can hold gas within the swim bladder using the pneumatic duct can vary with their size.  Understanding these pathways and how they may change with life stage is critical to accurately producing criteria for safe downstream passage of Sturgeon.

Sturgeon could be studied in several other ways to gain an understanding of their vulnerability to rapid decompression.  Cutting-edge imagery (X-ray radiographs and computed tomography [CT] scans) can be deployed to examine the Sturgeon, and has been used widely during fisheries research at PNNL.  For example, using radiographs of juvenile White Sturgeon, Brown et al. (2013a) found a great deal of variability among swim bladder sizes of fish of the same age.  They found that only about half of the Sturgeon had gas in their swim bladders at 91 days post-hatch and that those that were inflated had a wide variety of gas within their swim bladders and digestive tracts.  Another way to quantify the amount of undissolved gas in Sturgeon is not only to attain X-ray radiographs, but to also employ CT scanners (available at PNNL) while passing water over the fish's gills.  This would allow the volume of gas to be determined among multiple Sturgeon prior to exposures reflecting rapid passage through hydro facilities.  This technique has also been used to determine the volume of swim bladders in juvenile Eels, and also to measure the large amounts of bubbles discovered in their digestive tracts (R. S. Brown; PNNL, personal communication).

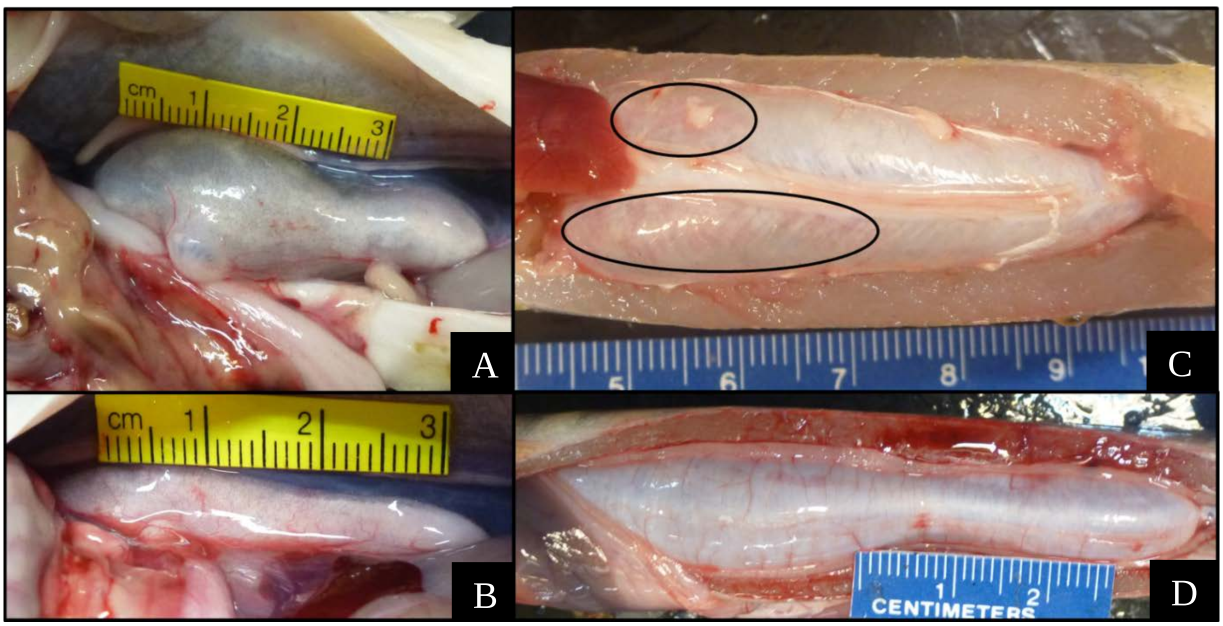


**Figure 4**.   The variability in swim bladder inflation for juvenile White Sturgeon is shown both (A) inflated and (B) deflated.  The swim bladder of a Walleye with the rete circled in black (C) and the fully inflated swim bladder of a Tiger Muskie (D).

Another way to understand barotrauma vulnerabilities would be to place Sturgeon in a very large acrylic pressure chamber system.  Researchers at PNNL have used a 4 m high cylindrical pressure chamber to study juvenile Salmon, and it also would be a good test bed for examining the vulnerability of Sturgeon to barotrauma.  The test chamber can replicate the pressure equivalent from surface pressure to simulated depths of over 20 m.  Fish placed in hypo/hyperbaric chambers like this can be exposed to simulated pressures similar to those they would choose in the wild.  Doing this could perhaps allow the fish to act more naturally while being monitored, and their depth of neutral buoyancy to be determined.

Another consideration is the possibility that a Sturgeon's diet may influence the level of gas in its digestive tract.  Research could be conducted to determine whether a salmon-based pellet diet, or other commonly used diet at Sturgeon hatcheries, influence the amount of gas found in the digestive tract of

Sturgeon when compared to Sturgeon that have a more natural diet. The compositions of the gas in the digestive tract and in the swim bladder could be determined using gas chromatography-mass spectrometry and compared to the composition of air. The comparison could indicate whether the Sturgeon are using a rete instead of solely gulping gas from the surface of the water; however, based on the research reported here it appears that the rete plays little if any role among young White Sturgeon (similar to Fange 1983).

Quantifying the volume of gas in the Sturgeon would be done using a net pen within a large water body. Sturgeon of a wide size range could be placed inside a large deep net pen that would be deployed with multiple video cameras (and acoustic cameras if needed) monitoring the entire length of the net pen. The Sturgeon could be provided with food while residing in the pen. After the Sturgeon appeared accustomed to the net pen, it could be very slowly raised upward in the water column (similar to what is described by Brown et al. 2014). The depth at which the fish start to behave like neutrally buoyant fish could be determined (Pflugrath et al. 2012). Any gas that the fish expel would be collected using a funnel type structure to collect gas expelled from the fish. The funnel for the system could cover all of the net pen's surface to quantify the amount of gas that is expelled. It may need to be employed just before the fish are lifted to the surface to make sure that bubbles would not form and negatively influence results. The fish would also be euthanized when it reached the surface and the gas in the swim bladder and the digestive tract could be collected. Using methods of Pflugrath et al. (2012), the depth at which the fish was neutrally buoyant can be determined.

# 5.0 Conclusion

To develop criteria for robust relationships between RPC and fish injuries and mortality for Tiger Muskie and Walleye, it is necessary to test a total of 300 fish for each species, evenly spaced across a range of RPCs, Also, Northern Pike and Lake Sturgeon should be tested to validate the use of Tiger Muskie and White Sturgeon as surrogates. This research revealed that juvenile White Sturgeon did not become neutrally buoyant within a pressure chamber at acclimation pressures near surface pressure and equivalent to greater depths.

Due to these realities, it is critical to develop noninvasive methods of quantifying the volume of gas within Sturgeon in locations where they could be exposed to rapid pressure decompression. This is necessary for accurate development of a robust criteria relationship between rapid pressure change and mortality or mortal injury. Although ultrasound units have been used for decades, it is possible that newer, very accurate, digital ultrasound units could be used to determine the size and volume of the swim bladder or any other bubbles while the fish are in the chamber prior to exposure to rapid decompression. Experimentation is currently being planned with these units by researchers at PNNL.

Also, testing fish in tall columns of water may be fruitful. Pilot-scale work (as part of a different project) that has been done at PNNL with small White Sturgeon has shown that they mainly just swim up and down in the column. However, adding sources of dark refuge within this test unit may allow experiments to be done. This is because Sturgeon and other benthic fish are known to move into draft tubes of hydro dams when turbine units are turned off. Also, benthic fish like Sturgeon may generally prefer artificial cover. Such research would allow the pressure the fish are experiencing to be jogged up and down to determine the pressure at which they are neutrally buoyant. Using net pens in the forebays of dams or lakes may also be helpful, but more difficult and expensive than laboratory work.

This research has provided some insight into the pathways of injury among Sturgeon exposed to rapid decompression. Some barotrauma pathways were determined by exposing them to STP without knowing the volume of gas found in their swim bladders prior to STP. Several ways to understand the vulnerability of Sturgeon to barotrauma have been detailed in this document. These actions, using a combination of laboratory and possibly field experimentation, could be used to determine the relationship between rapid decompression and barotrauma injuries.

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# Appendix A

# Estimated Sample Sizes for LRP Mortality Studies of Tiger Muskie and Walleye

# Appendix A

# Estimated Sample Sizes for LRP Mortality Studies of Tiger Muskie and Walleye

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5 March 2015

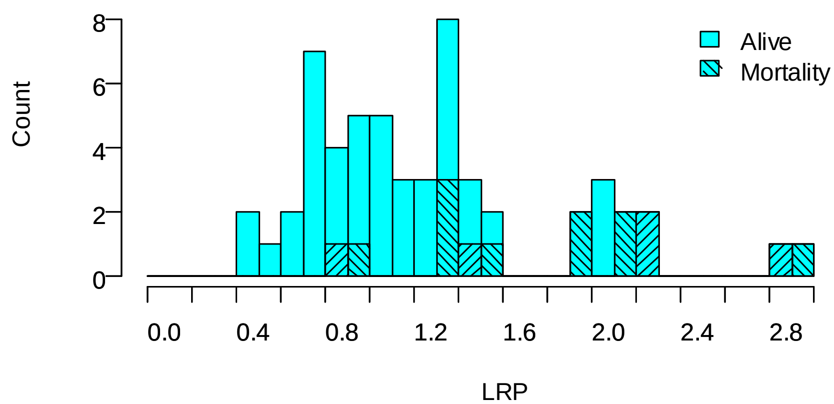
## Introduction

The goal of this analysis is to assess the sample sizes required to obtain a maximum standard error on a logged ratio pressure (LRP) regression on expected mortality. The sample sizes provided in this document are based on the results from preliminary mortality studies conducted in 2014. Future studies should be incorporated into the methods described below to improve the results. These sample sizes apply only to the relationship of LRP to expected mortality, and do not incorporate other information, such as the rate of the pressure change.

## Data

In order to estimate the required sample sizes for future studies, the current data were analyzed. Figure 1 shows histograms of the tested LRP levels for each species, and the outcomes at each level. The final study will require a spread of LRP values, with no gaps.

a. Walleye




b. Tiger Muskie

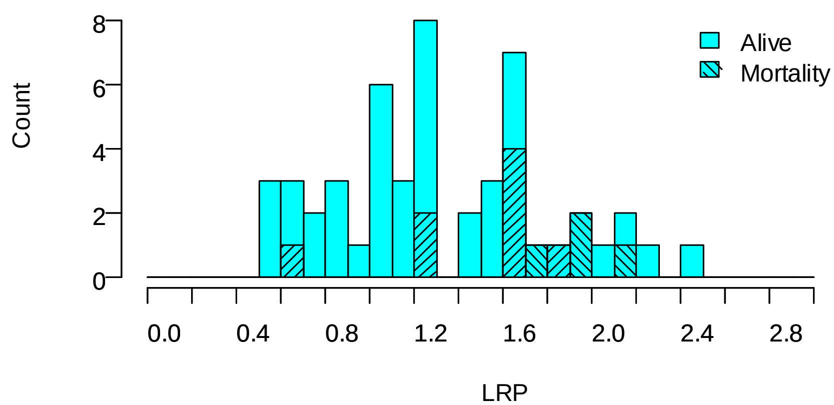



**Figure 1**. Histograms of the logged Ratio Pressure test in the a) Walleye and b) Tiger Muskie studies. Shaded areas indicate the number of mortalities that occurred at each level of LRP.

## Analysis

Analysis of each species used a general linear model (GLM) regression of the LRP on the binomial outcome of mortality (1 = dead, 0 = alive), using a logit link:

$$\log\left(\frac{p_i}{1-p_i}\right) = \alpha + \beta * \text{LRP}_i$$

where $p_i$ = probability of mortality for the $i^{th}$ fish, and

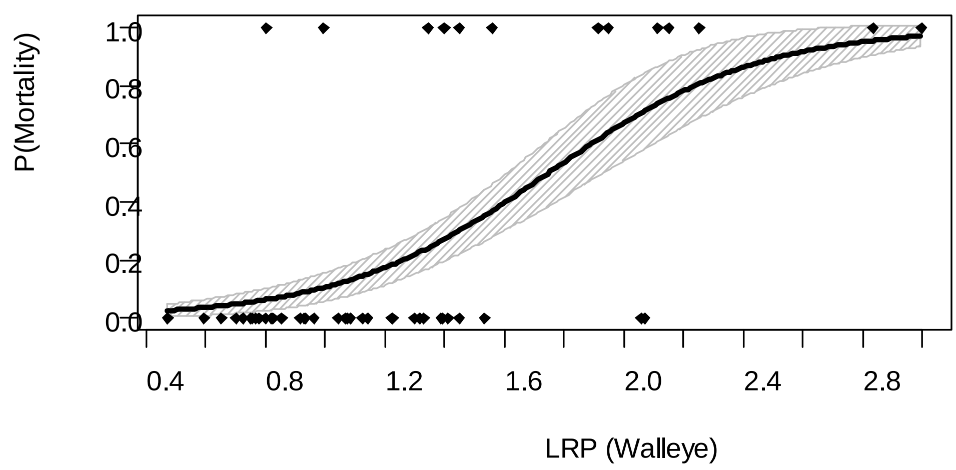
$\text{LRP}_i$ = $\log(\frac{acclimation\ pressure_i}{nadir\ pressure_i})$.

The fitted models (Table 1), were then used to estimate the probabilities of mortality for a given LRP. The fitted mortality curves versus LRP are illustrated in Figure 2.

**Table 1**. Model coefficients from a GLM regression (logit link) on mortality for each species.

| Species | | Coef. Est. | SE | Z-value | P(>\|Z\|) |
|---|---|---|---|---|---|
| Walleye | Intercept | -4.9565 | 1.2201 | -4.062 | < 0.0001 |
| | LRP | 2.8380 | 0.8086 | 3.510 | 0.0004 |
| Tiger Muskie | Intercept | -3.9263 | 1.2789 | -3.070 | 0.0021 |
| | LRP | 1.9573 | 0.8185 | 2.391 | 0.0168 |

Walleye




a) Tiger Muskie

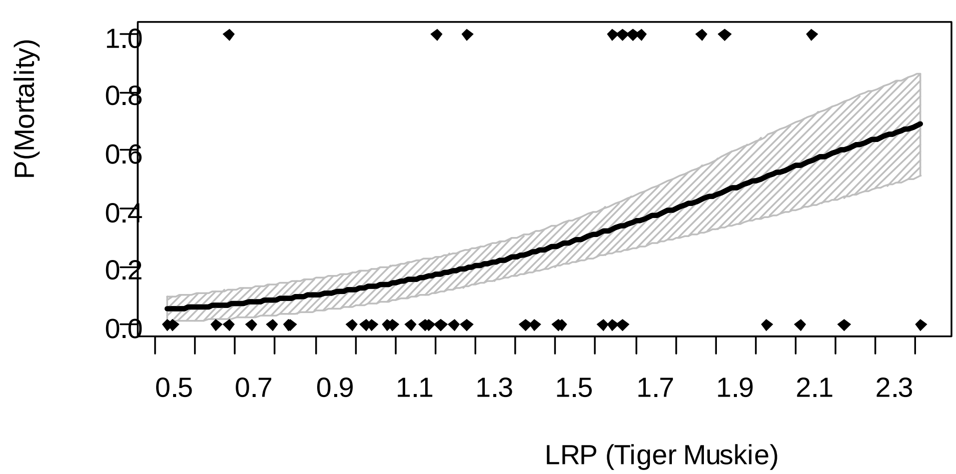


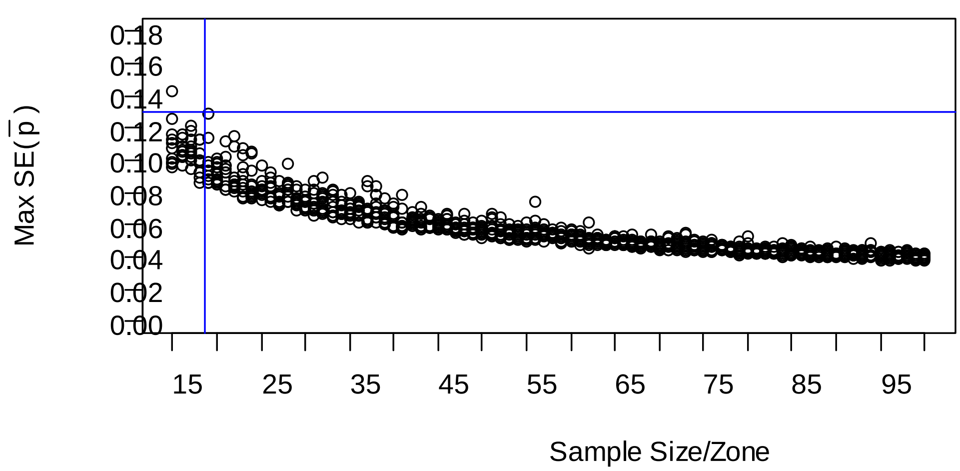

**Figure 2**. Fitted LRP mortality curves using the 2014 test data for a) Walleye and b) Tiger Muskie. Shaded area shows 1 standard error around a point on the line. Points are the observed mortality outcomes for each study.

## Simulation of expected standard errors for a given sample size

Assuming that the range of tested LRP values is what will be used for future studies, the observed range (approximately $0 \leq LRP \leq 2.8$) was divided into three equal segments. This stratification was used to ensure a uniform spread of test values. Within each segment, an equal number of samples of LRP values were picked, using a uniform distribution. For each LRT value, the probability of mortality was estimated using the fitted model for the appropriate species, then a binomial "flip of the coin" was performed to determine whether a mortality occurred or not.

Using the simulated data, a new regression model was fit and the maximum standard error on the curve was estimated. This process was repeated 9 more times at each sample size. The results for each species are shown in Figure 3. For each model, the simulated maximum standard error decreases as the sample size increases, and indicates a smooth downward trend, as expected with additional test fish. As a reality check, blue lines in each plot show the observed maximum standard error and sample size from the 2014 LRP mortality studies. For both species, precision improvements become small beyond 100 fish/zone (300 fish total).

a) Walleye




b) Tiger Muskie

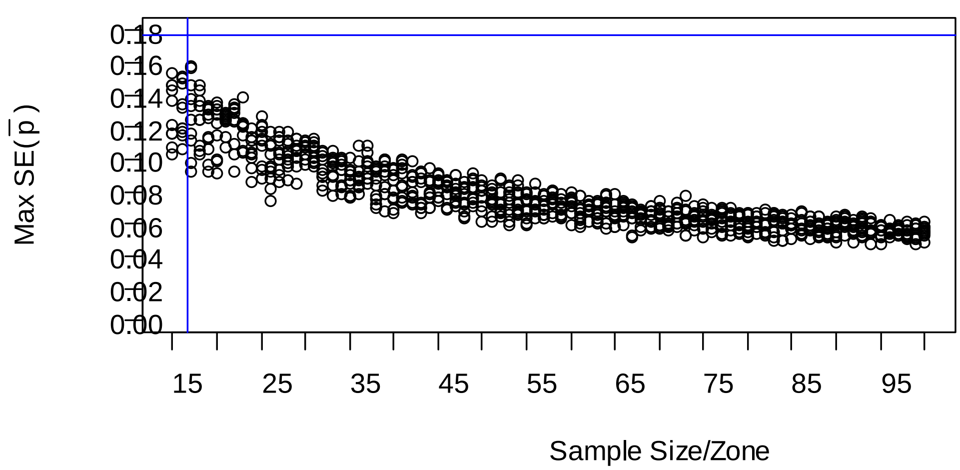



**Figure 3**. Simulated results of sample sizes of 15-100 per zone, for 3 zones, for a) Walleye and b) Tiger Muskie. Total fish required will be 3 times the sample size shown (i.e., 15 = 45 fish total). The blue lines indicate the observed value of maximum standard error on the existing regression curves for the 2014 LRP mortality studies.

A smooth polynomial line was fitted to the simulated maximum standard error values for each species (Figure 4). Table 2 has the zone definitions used in these simulations. When planning future studies, the only requirement is an even distribution of LRP values over the range tested.

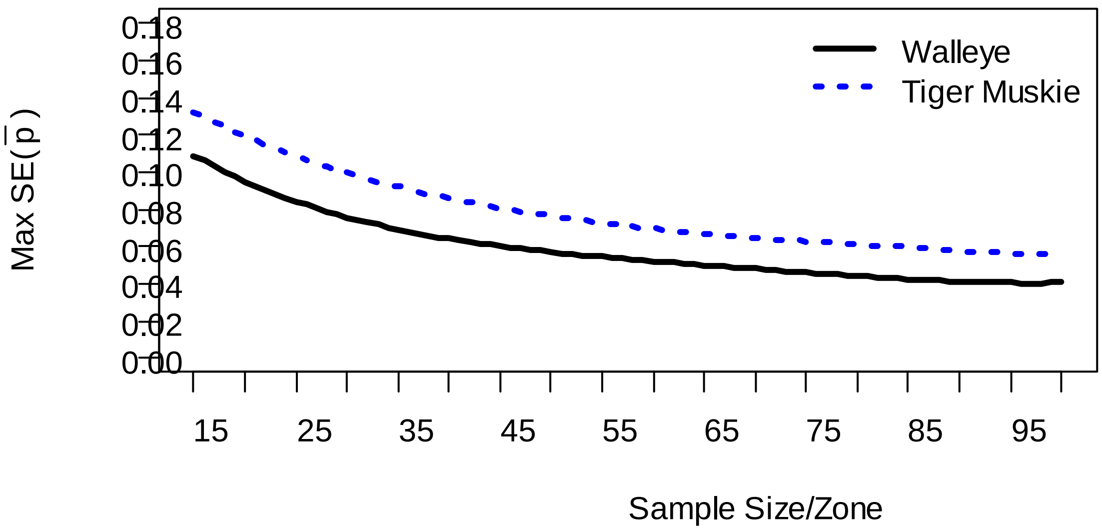



**Figure 4**. Polynomial line of the simulated results of sample sizes of 15-100 per zone, for three zones, for the Walleye (solid line) and Tiger Muskie (dashed line). Total fish required will be 3 times the sample size shown (e.g., 15 = 45 fish total).

**Table 2**. LRP zones used in this simulation.

| | Logged Ratio Pressure | | |
|---|---|---|---|
| Species | Zone 1 | Zone 2 | Zone 3 |
| Walleye | 0.5-1.3 | 1.3-2.2 | 2.2-3.0 |
| Tiger Muskie | 0.5-1.2 | 1.2-1.8 | 1.8-2.4 |

## Conclusion

A total sample size of approximately 300 fish per species is recommended in constructing LRP mortality curves. It is **important** to note that the precision calculations are based on the assumption of an even distribution of test fish across the LRP range. Regression results are sensitive to the distribution of the independent variable (e.g., LRP) used in model fitting. Realized distributions other than uniform may result in precision results different than projected in this report.

# Appendix B

# Individual Fish Data Used to Determine Adequate Sample Sizes for a Robust Relationship Curve between LRP and Mortal Injury

# Appendix B

## Individual Fish Data Used to Determine an Adequate Sample Sizes for a Robust Relationship Curve between LRP and Mortal Injury

| Fish Number | Species | Length (mm) | Weight (g) | Acclimation Pressure (psia) | Neutrally Buoyancy @ (psia) | Ratio of Pressure Change | Nadir | Rate of Pressure Change | Mortal Injury (Y=1, N=0) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Walleye | 275 | 185.8 | 21.2 | 19 | 2.92 | 6.5 | 99 | 0 |
| 2 | Walleye | 298 | 231.1 | 21.2 | 21.2 | 2.49 | 8.5 | 157 | 0 |
| 3 | Walleye | 191 | 49.3 | 21.2 | 17 | 2.54 | 6.7 | 147 | 0 |
| 4 | Walleye | 224 | 92.1 | 21.2 | 19 | 2.84 | 6.7 | 147 | 0 |
| 5 | Walleye | 238 | 111.3 | 21.2 | 17 | 2.62 | 6.5 | 121 | 0 |
| 6 | Walleye | 239 | 118.8 | 21.2 | 20 | 3.08 | 6.5 | 121 | 0 |
| 7 | Walleye | 184 | 54.5 | 21.2 | 18 | 7.83 | 2.3 | 318 | 0 |
| 8 | Walleye | 244 | 124.5 | 21.2 | 19 | 8.26 | 2.3 | 318 | 1 |
| 9 | Walleye | 231 | 96.9 | 21.2 | 19 | 4.63 | 4.1 | 130 | 0 |
| 10 | Walleye | 230 | 100.0 | 21.2 | 15 | 3.66 | 4.1 | 130 | 0 |
| 11 | Walleye | 229 | 103.6 | 21.2 | 17 | 3.78 | 4.5 | 201 | 0 |
| 12 | Walleye | 252 | 129.5 | 21.2 | 18 | 4.00 | 4.5 | 201 | 0 |
| 13 | Walleye | 228 | 85.2 | 21.2 | 15 | 2.17 | 6.9 | 202 | 0 |
| 14 | Walleye | 239 | 119.7 | 21.2 | 14.7 | 2.13 | 6.9 | 202 | 0 |
| 15 | Walleye | 212 | 71.5 | 21.2 | 19 | 2.35 | 8.1 | 215 | 0 |
| 16 | Walleye | 215 | 84.0 | 21.2 | 18 | 2.22 | 8.1 | 215 | 0 |
| 17 | Walleye | 239 | 109.5 | 21.2 | 18 | 2.95 | 6.1 | 103 | 0 |
| 18 | Walleye | 209 | 78.4 | 21.2 | 18 | 2.95 | 6.1 | 103 | 0 |
| 19 | Walleye | 223 | 74.7 | 21.2 | 17 | 2.27 | 7.5 | 179 | 0 |
| 20 | Walleye | 241 | 108.3 | 21.2 | 19 | 2.53 | 7.5 | 179 | 0 |
| 21 | Walleye | 198 | 57.4 | 21.2 | 20 | 3.13 | 6.4 | 203 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 22 | Walleye | 232 | 110.4 | 21.2 | 20 | 3.13 | 6.4 | 203 | 0 |
| 23 | Walleye | 222 | 87.9 | 21.2 | 16 | 4.10 | 3.9 | 98 | 0 |
| 24 | Walleye | 248 | 139.3 | 21.2 | 16.6 | 4.26 | 3.9 | 98 | 1 |
| 25 | Walleye | 230 | 117.1 | 21.2 | 16.1 | 4.03 | 4 | 112 | 0 |
| 26 | Walleye | 225 | 96.1 | 21.2 | 19 | 4.75 | 4 | 112 | 1 |
| 27 | Walleye | 232 | 93.6 | 21.2 | 16.9 | 2.06 | 8.2 | 90 | 0 |
| 28 | Walleye | 200 | 70.4 | 21.2 | 16.9 | 2.06 | 8.2 | 90 | 0 |
| 29 | Walleye | 205 | 73.5 | 21.2 | 17 | 1.60 | 10.6 | 87 | 0 |
| 30 | Walleye | 209 | 78.7 | 21.2 | 17 | 1.60 | 10.6 | 87 | 0 |
| 31 | Walleye | 206 | 76.2 | 21.2 | 19 | 9.50 | 2 | 139 | 1 |
| 32 | Walleye | 210 | 72.1 | 21.2 | 19 | 9.50 | 2 | 139 | 1 |
| 33 | Walleye | 213 | 78.1 | 21.2 | 18 | 3.83 | 4.7 | 110 | 1 |
| 34 | Walleye | 300 | 99.7 | 21.2 | 18 | 3.83 | 4.7 | 110 | 1 |
| 35 | Walleye | 193 | 56.6 | 21.2 | 17 | 3.40 | 5 | 119 | 0 |
| 36 | Walleye | 216 | 76.0 | 21.2 | 17 | 3.40 | 5 | 119 | 0 |
| 37 | Walleye | 227 | 94.8 | 21.2 | 19 | 4.04 | 4.7 | 112 | 1 |
| 38 | Walleye | 257 | 139.6 | 21.2 | 20 | 4.26 | 4.7 | 112 | 0 |
| 39 | Walleye | 243 | 109.8 | 21.2 | 17 | 17.00 | 1 | 129 | 1 |
| 40 | Walleye | 178 | 40.9 | 21.2 | 20 | 20.00 | 1 | 129 | 1 |
| 41 | Walleye | 224 | 86.0 | 21.2 | 19 | 3.73 | 5.1 | 203 | 0 |
| 42 | Walleye | 202 | 56.9 | 21.2 | 19 | 3.73 | 5.1 | 203 | 0 |
| 43 | Walleye | 238 | 110.4 | 21.2 | 14.7 | 2.01 | 7.3 | 120 | 0 |
| 44 | Walleye | 238 | 166.2 | 21.2 | 14 | 1.92 | 7.3 | 120 | 0 |
| 45 | Walleye | 229 | 101.7 | 21.2 | 20 | 2.70 | 7.4 | 173 | 1 |
| 46 | Walleye | 205 | 72.9 | 21.2 | 16.5 | 2.23 | 7.4 | 173 | 1 |
| 47 | Walleye | 225 | 94.9 | 21.2 | 19 | 7.92 | 2.4 | 130 | 0 |
| 48 | Walleye | 293 | 210.0 | 21.2 | 19 | 7.92 | 2.4 | 130 | 0 |
| 49 | Walleye | 264 | 152.0 | 21.2 | 17 | 2.15 | 7.9 | 200 | 0 |
| 50 | Walleye | 215 | 68.8 | 21.2 | 18 | 2.28 | 7.9 | 200 | 0 |
| 51 | Walleye | 304 | 272.4 | 21.2 | 14.7 | 7.00 | 2.1 | 134 | 1 |
| 52 | Walleye | 289 | 219.3 | 21.2 | 15.2 | 1.81 | 8.4 | 82 | 0 |

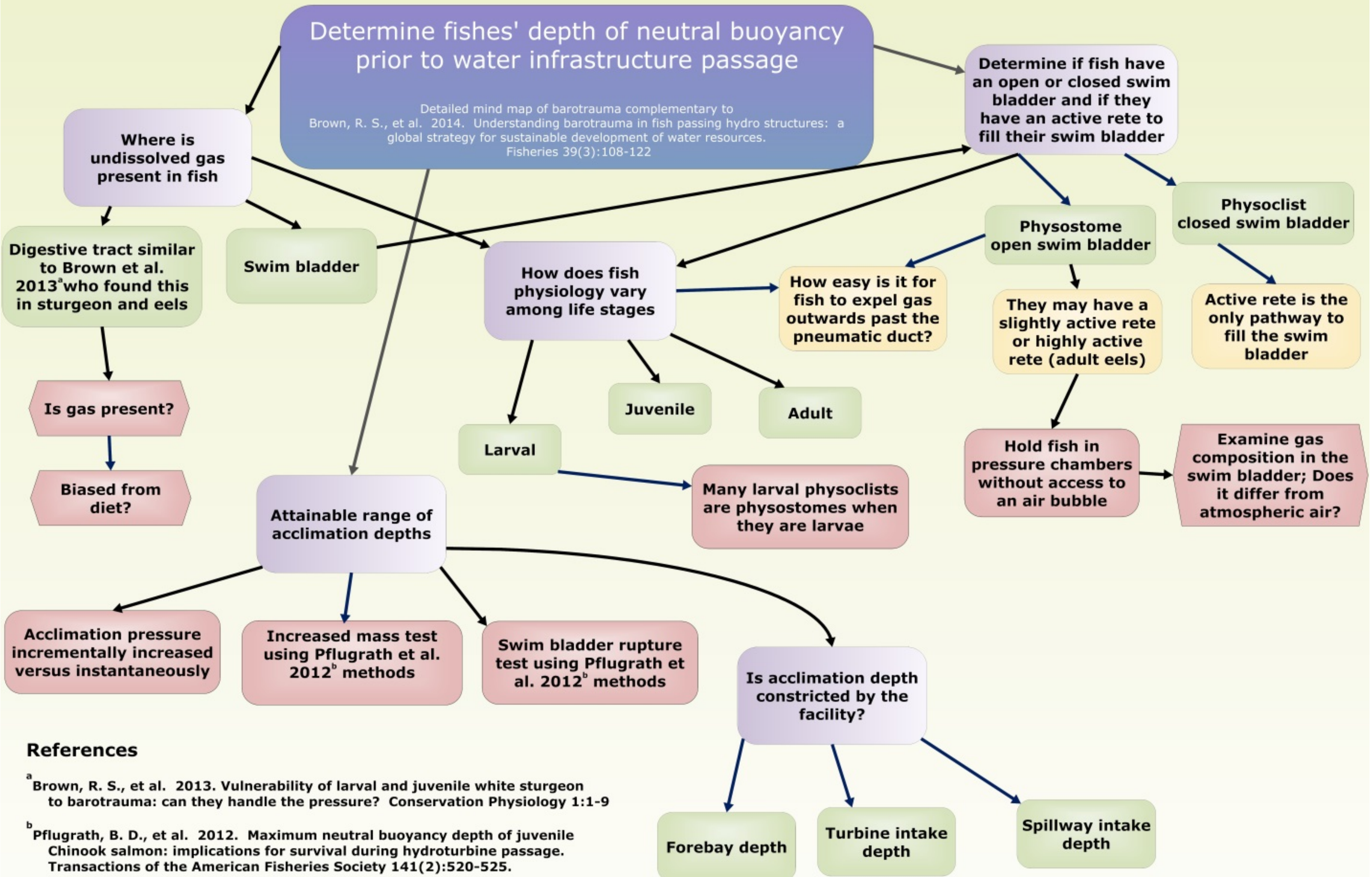
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 53 | Walleye | 319 | 260.6 | 21.2 | 14.9 | 6.77 | 2.2 | 169 | 1 |
| 54 | Walleye | 304 | 259.5 | 21.2 | 18 | 2.12 | 8.5 | 131 | 0 |
| 55 | Walleye | 310 | 255.4 | 21.2 | 18 | 8.57 | 2.1 | 188 | 1 |
| 56 | Walleye | 319 | 269.2 | 21.2 | 18 | 2.90 | 6.2 | 135 | 0 |
| 1 | Tiger Muskie | 162 | 22.0 | 21.2 | 21.2 | 4.51 | 4.7 | 106 | 0 |
| 2 | Tiger Muskie | 136 | 12.9 | 21.2 | 21.2 | 4.51 | 4.7 | 106 | 0 |
| 3 | Tiger Muskie | 141 | 13.7 | 21.2 | 16.9 | 1.72 | 9.8 | 95 | 0 |
| 4 | Tiger Muskie | 160 | 21.8 | 21.2 | 16.9 | 1.72 | 9.8 | 95 | 0 |
| 5 | Tiger Muskie | 164 | 21.5 | 21.2 | 21.2 | 1.98 | 10.7 | 183 | 1 |
| 6 | Tiger Muskie | 122 | 8.2 | 21.2 | 21.2 | 1.98 | 10.7 | 183 | 0 |
| 7 | Tiger Muskie | 159 | 21.2 | 21.2 | 21.2 | 5.30 | 4 | 197 | 0 |
| 8 | Tiger Muskie | 130 | 9.5 | 21.2 | 21.2 | 5.30 | 4 | 197 | 1 |
| 9 | Tiger Muskie | 147 | 17.1 | 21.2 | 21.2 | 3.37 | 6.3 | 136 | 0 |
| 10 | Tiger Muskie | 124 | 13.5 | 21.2 | 21.2 | 3.37 | 6.3 | 136 | 0 |
| 11 | Tiger Muskie | 153 | 18.7 | 21.2 | 21.2 | 2.79 | 7.6 | 117 | 0 |
| 12 | Tiger Muskie | 126 | 10.0 | 21.2 | 21.2 | 2.79 | 7.6 | 117 | 0 |
| 13 | Tiger Muskie | 134 | 10.4 | 21.2 | 21.2 | 5.44 | 3.9 | 120 | 1 |
| 14 | Tiger Muskie | 165 | 22.8 | 21.2 | 21.2 | 5.44 | 3.9 | 120 | 1 |
| 15 | Tiger Muskie | 127 | 10.2 | 21.2 | 21.2 | 2.99 | 7.1 | 123 | 0 |
| 16 | Tiger Muskie | 159 | 21.1 | 21.2 | 21.2 | 2.99 | 7.1 | 123 | 0 |
| 17 | Tiger Muskie | 149 | 20.9 | 21.2 | 21.2 | 5.17 | 4.1 | 200 | 1 |
| 18 | Tiger Muskie | 131 | 11.1 | 21.2 | 21.2 | 5.17 | 4.1 | 200 | 0 |
| 19 | Tiger Muskie | 133 | 13.0 | 21.2 | 21.2 | 2.30 | 9.2 | 79 | 0 |
| 20 | Tiger Muskie | 164 | 21.7 | 21.2 | 21.2 | 2.30 | 9.2 | 79 | 0 |
| 21 | Tiger Muskie | 237 | 76.3 | 21.2 | 17 | 2.10 | 8.1 | 151 | 0 |
| 22 | Tiger Muskie | 190 | 39.3 | 21.2 | 21.2 | 2.21 | 9.6 | 203 | 0 |
| 23 | Tiger Muskie | 237 | 71.6 | 21.2 | 16 | 1.70 | 9.4 | 78 | 0 |
| 24 | Tiger Muskie | 193 | 41.7 | 21.2 | 17 | 2.83 | 6 | 163 | 0 |
| 25 | Tiger Muskie | 233 | 74.7 | 21.2 | 21.2 | 3.12 | 6.79 | 193 | 0 |
| 26 | Tiger Muskie | 259 | 103.6 | 21.2 | 19.6 | 1.92 | 10.21 | 145 | 0 |
| 27 | Tiger Muskie | 200 | 45.1 | 21.2 | 19 | 8.26 | 2.3 | 149 | 0 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 28 | Tiger Muskie | 183 | 35.7 | 21.2 | 20 | 4.55 | 4.4 | 87 | 0 |
| 29 | Tiger Muskie | 180 | 27.5 | 21.2 | 16.2 | 2.95 | 5.5 | 82 | 0 |
| 30 | Tiger Muskie | 204 | 43.7 | 21.2 | 21.2 | 4.16 | 5.1 | 131 | 0 |
| 31 | Tiger Muskie | 224 | 60.2 | 21.2 | 14.2 | 6.45 | 2.2 | 131 | 1 |
| 32 | Tiger Muskie | 204 | 48.0 | 21.2 | 21.2 | 5.05 | 4.2 | 188 | 0 |
| 33 | Tiger Muskie | 206 | 49.7 | 21.2 | 21.2 | 3.48 | 6.1 | 94 | 0 |
| 34 | Tiger Muskie | 223 | 64.6 | 21.2 | 20.8 | 2.31 | 9 | 133 | 0 |
| 35 | Tiger Muskie | 190 | 39.1 | 21.2 | 16 | 3.33 | 4.8 | 151 | 1 |
| 36 | Tiger Muskie | 214 | 54.9 | 21.2 | 18.6 | 2.70 | 6.9 | 135 | 0 |
| 37 | Tiger Muskie | 210 | 51.5 | 21.2 | 21.2 | 9.22 | 2.3 | 162 | 0 |
| 38 | Tiger Muskie | 216 | 54.0 | 21.2 | 19 | 3.58 | 5.3 | 124 | 0 |
| 39 | Tiger Muskie | 232 | 78.3 | 21.2 | 21.1 | 5.55 | 3.8 | 186 | 1 |
| 40 | Tiger Muskie | 219 | 59.4 | 21.2 | 21.2 | 3.37 | 6.3 | 225 | 0 |
| 41 | Tiger Muskie | 247 | 93.4 | 21.2 | 17 | 8.50 | 2 | 157 | 1 |
| 42 | Tiger Muskie | 210 | 50.3 | 21.2 | 20 | 4.26 | 4.7 | 88 | 0 |
| 43 | Tiger Muskie | 216 | 60.5 | 21.2 | 16.5 | 3.24 | 5.1 | 143 | 0 |
| 44 | Tiger Muskie | 217 | 58.6 | 21.2 | 17 | 3.27 | 5.2 | 149 | 0 |
| 45 | Tiger Muskie | 226 | 65.2 | 21.2 | 21.2 | 11.16 | 1.9 | 150 | 0 |
| 46 | Tiger Muskie | 213 | 51.2 | 21.2 | 20.5 | 7.59 | 2.7 | 127 | 0 |
| 47 | Tiger Muskie | 202 | 42.4 | 21.2 | 21.2 | 6.84 | 3.1 | 204 | 1 |
| 48 | Tiger Muskie | 232 | 68.1 | 21.2 | 21.2 | 6.84 | 3.1 | 204 | 1 |
| 49 | Tiger Muskie | 218 | 57.4 | 21.2 | 21.2 | 3.59 | 5.9 | 127 | 0 |
| 50 | Tiger Muskie | 225 | 66.2 | 21.2 | 21.2 | 3.59 | 5.9 | 127 | 1 |

# Appendix C

## Precursors to Attaining the Range of Possible Depths of Neutral Buoyancy in Lab-Based Studies: Critical Research for Appropriately Treating Fish Prior to Rapid Decompression Testing to Determine Their Vulnerability to Barotrauma

**Precursors to attaining the range of possible depths of neutral buoyancy in lab-based studies: critical research for appropriately treating fish prior to rapid decompression testing to determine their vulnerability to barotrauma**





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[b] Pflugrath, B. D., et al. 2012. Maximum neutral buoyancy depth of juvenile Chinook salmon: implications for survival during hydroturbine passage. Transactions of the American Fisheries Society 141(2):520-525.

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