Appendix P

Effects of Tidal Turbine Noise on Fish Hearing and Tissues
Pacific Northwest National Laboratory

U.S. DEPARTMENT OF ENERGY

PNNL-20786

Prepared for the U.S. Department of Energy
under Contract DE-AC05-76RL01830

Effects of Tidal Turbine Noise on Fish Hearing and Tissues

Draft Final Report

Environmental Effects of Marine and Hydrokinetic Energy

MB Halvorsen
TJ Carlson
AE Copping

September 2011

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Pacific Northwest National Laboratory
Sequim, Washington 98382

Abstract

Snohomish Public Utility District #1 plans to deploy two 6 meter OpenHydro tidal turbines in Admiralty Inlet in Puget Sound, under a FERC pilot permitting process. Regulators and stakeholders have raised questions about the potential effect of noise from the turbines on marine life. Noise in the aquatic environment is known to be a stressor to many types of aquatic life, including marine mammals, fish and birds. Marine mammals and birds are exceptionally difficult to work with for technical and regulatory reasons. Fish have been used as surrogates for other aquatic vertebrates as they have similar auditory structures. For this study, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) were used as the experimental animal. Plans exist for prototype tidal turbines to be deployed into their habitat. Noise is known to affect fish in many ways, such as causing a threshold shift in auditory sensitivity or tissue damage. The characteristics of noise, its spectra and level, are important factors that influence the potential for the noise to injure fish. For example, the frequency range of the tidal turbine noise includes the audiogram (frequency range of hearing) of most fish, and the noise level of 160 dB re 1µPa SEL_{rms} 1 meter from the turbine is detectable by fish. This study was performed during FY 2011 to determine if noise generated by a 6-m diameter OpenHydro turbine might affect juvenile Chinook salmon hearing or cause barotrauma. Naturally spawning stocks of Chinook salmon that utilize Puget Sound are listed as threatened (http://www.nwr.noaa.gov/ESA-Salmon-Listings/Salmon-Populations/Chinook/CKPUG.cfm); the fish used in this experiment were hatchery raised and their populations are not in danger of depletion. After they were exposed to simulated tidal turbine noise, the hearing of juvenile Chinook salmon was measured and necropsies performed to check for tissue damage. Experimental groups were 1) noise exposed, 2) control (the same handling as treatment fish but without exposure to tidal turbine noise), and 3) baseline (never handled). Experimental results indicate that non-lethal, low levels of tissue damage may have occurred but that there were no effects of noise exposure on the auditory systems of the test fish.

This project was funded under the FY09 Funding Opportunity Announcement (FOA) to Snohomish PUD, in partnership with the University of Washington – Northwest National Marine Renewable Energy Center, the Sea Mammal Research Unit, and Pacific Northwest National Laboratory. The results of this study will inform the larger research project outcomes.

Acknowledgments

Thanks to Josh Myers for designing and assisting with implementation of the noise exposure electronics and measurement of the noise sound field in the exposure tank, and to Carmina Arimescu, Lara Aston, and Jennifer Elster for collection of hearing and tissue damage data. Many thanks to the PNNL wet lab staff for their assistance in getting the project off to a smooth running start and for their continual support throughout the experiment, especially Brett Romano and Rhonda Karls. We thank Anish Adhikari for exceptional care of the juvenile salmon over their residence in the wet lab. We extend our thanks to Brian Polagye and OpenHydro for providing PSD plots generated from their 6-m OpenHydro field recording. Finally, we thank Snohomish PUD and DOE for funding the project.

Acronyms and Abbreviations

AEP	auditory evoked potential
cm	centimeter(s)
dB	decibel
DOE	U.S. Department of Energy
EERE	DOE Office of Energy Efficiency and Renewable Energy
ERES	Environmental Risk Evaluation System
ESA	Endangered Species Act
FL	fork length
g	gram(s)
gal	gallon(s)
HAT	hearing assessment tube
hr	hour(s)
Hz	hertz
MHK	marine and hydrokinetic
µm	micrometer(s)
µl	microliter(s)
µPa	micropascal
ml	milliliter(s)
mm	millimeter(s)
NOAA	National Oceanic and Atmospheric Administration
PNNL	Pacific Northwest National Laboratory
PSD	power spectral density
RMS	root mean square
SEL	sound exposure level
SPL	sound pressure level
SD	standard deviation
SEM	standard error of the mean
TTS	temporary threshold shift
UW	University of Washington
WT	weight

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1.0 Introduction

Snohomish Public Utility District #1 plans to deploy two 6 meter OpenHydro tidal turbines in Admiralty Inlet in Puget Sound, under a FERC pilot permitting process. As renewable energy from marine and hydrokinetic devices are being developed and near deployment, the environmental effects that these devices may cause are being investigated. In particular, the level of interest from the public, regulatory, and scientific communities about the potential impacts of human-made (anthropogenic) underwater noise on aquatic animals has steadily increased over the last decade (Slabbekoorn et al., 2010). Marine mammals and birds are exceptionally difficult to work with for technical and regulatory reasons. Fish have been used as surrogates for other aquatic vertebrates as they have similar auditory structures. This report focuses on the potential effects on juvenile Chinook salmon, a threatened species, from noise generated by a tidal turbine.

This project was funded under the FY09 Funding Opportunity Announcement (FOA) to Snohomish PUD, in partnership with the University of Washington – Northwest National Marine Renewable Energy Center, the Sea Mammal Research Unit, and Pacific Northwest National Laboratory. The results of this study will inform the larger research project outcomes.

Sound propagates through the water as a compression and rarefaction wave. Sound can also propagate through the sea floor and can travel farther faster than the same sound in the water column. The energy in sound can cause tissue damage that can result from rapid changes in pressure, which directly affects the body gases and thus body tissues. Two types of changes in the state of gas within the body of a fish can lead to injury. Free gas in the swim bladder, or in natural bubbles in the blood and tissues of fishes, can expand and contract during changes in pressure that occur when a fish is exposed to sound. Such changes in the volume of free gas with pressure, if large enough, can cause tissue damage. In addition, simultaneously with changes in the volume of free gas, changes in the solubility of gas in the blood and tissues and other fluids can also occur, which leads to the formation of free gas in the arteries, veins, and organs of exposed fish.

The majority of fish species have a swim bladder that is critical for control of buoyancy. Changes in external pressure may cause rapid and substantial changes in the volume of the swim bladder, which stresses swim bladder tissue and may lead to tearing of the tissue and rupture of the swim bladder. A ruptured swim bladder compromises the fish's swimming performance, thereby increasing the risk for further injury or predation because it cannot maintain buoyancy and behave normally. In addition, the rapid and large changes in swim bladder volume may damage nearby tissues by exerting higher pressure on them when they are compressed between the swim bladder and the relatively inelastic body wall of the fish.

In addition, fishes have dissolved gas in their blood and body tissues at the same tensions as that in the water they inhabit. At decompression, the amount of gas that can remain in solution decreases. The gas that leaves solution forms bubbles in the blood and body tissues. The presence of these bubbles increases the pressure in the vessels and can cause their rupture. Gas bubbles in a fish's circulatory system can disrupt function or damage vital organs such as the heart, gills, kidney, and brain. The most severe effects, such as bubbles in the gills or heart, may result in immediate mortality.

Fish can suffer various types of tissue damage from exposure to sound (e.g., ruptured swim bladder or fin hematoma). To determine the effects of tidal turbines and other noise sources on aquatic organisms, it is essential to understand the acoustic characteristics of the noise they generate. When assessing the potential harm, noise characteristics of importance include the frequency content, peak pressure levels, and total energy. Furthermore, the propagation of sound and environmental factors such as bathymetry, bottom substrate type, water temperature, and salinity are some factors can affect the exposure consequences of the fish from sound.

Almost all fish can hear infrasonic and low-frequency sounds that can range from around 15 Hz up to 1 kHz. Fish live in acoustically complex environments and are well equipped to make use of the sound they sense to increase their likelihood of survival (Fay, 2000; Zeddies et al., 2010). Fish use audition for the same purposes as mammals—to detect, locate, and identify their surroundings, such as location and identification of conspecifics, predators, and prey, and much more.

Sounds that are intense and/or of long duration have been shown to affect the auditory system of fish (Amoser et al., 2003; Halvorsen et al., 2009). If the auditory system is affected by sound, it is often expressed as a temporary shift in hearing threshold (McCauley et al., 2001; Smith et al., 2006; Popper et al., 2007; Halvorsen et al., 2009). Many types of anthropogenic sources, such as shipping, boat engines, some sonars, and wind and water turbines, produce low-frequency sounds. Any of these sources could cause damage to or mask fish auditory sensitivity, especially if fish were motivated by other factors, such as the presence of food, to remain in an area ensonified by continuous sound sources. A temporary loss of sensory function, like hearing, could have implications for survival because the fish may be at a decreased capacity to detect predators, prey, and/or conspecifics. Furthermore, for fish, temporary threshold shift (TTS) is considered the onset of injury from noise exposure (Popper et al., 2006) by many regulatory authorities. This approach to exposure criteria for noise and regulation of noise exposure follows notions for marine mammals (Southall et al., 2007).

Of concern is the effect from the turbine generated noise on nearby organisms of ecological importance. Juvenile Chinook salmon (*Oncorhynchus tshawytscha*) was the fish species selected for response testing to tidal turbine noise because they are an *Endangered Species Act of 1976* (ESA) -listed species, and they travel through Admiralty Inlet in Puget Sound both as juveniles and adults. Furthermore, marine hydrokinetic turbines generate noise energy that falls within the audible portion of the frequency range for most fishes (Atema et al., 1988).

This study focused on measuring the auditory and tissue effects on fish from simulated tidal turbine noise that was presented continuously for 24 hours around an SPL_{rms} of 159 dB re 1 μPa, which represents what is believed to be a worst-case exposure scenario for juvenile salmon. That level corresponds to the source level (defined as 1 m from the sound source) for a prototype turbine estimated from measurements of an operating 6 m turbine (Brian Polayge, University of Washington, personal communication, March 2011). After exposure to the noise, fish were assessed at four different time points for tissue damage and for changes in hearing sensitivity.

Fish can experience a temporary change in hearing sensitivity, which is called a temporary threshold shift (TTS). The shift may not affect the total frequency range of hearing equally but may be localized on a smaller frequency band within the total audible range of the fish. Not all fish have the same hearing sensitivity. In fact, the range in hearing capability across fish species varies a great deal (Atema et al., 1988). In this study, we used a noninvasive neurophysiological technique called auditory evoked

potential (AEP) to measure the threshold of hearing for specific frequencies across the auditory range of our test fish.

The tissue damage exams consisted of external examination of the whole fish followed by necropsy to inspect for the occurrence and severity of 72 different external and internal injuries known to occur in fish exposed to noise. Depending on the severity of tissue damage, the physiological cost to the fish could range from mortality to a complete recovery without even a short-term impact on behavior or physiological function. Recent advances in the assessment of tissue damage (barotrauma) have resulted in an injury evaluation method that is very sensitive to detection and severity assessment for fish tissue damage cause by sound. The model developed to translate injury observations into a quantitative measure of fish response to sound exposure is called the Fish Index of Trauma (FIT) (Carlson et al., 2011; Halvorsen et al., 2011 In press). The FIT model was applied to the data obtained for this study to quantify observed levels of tissue damage in juvenile Chinook salmon from long-duration exposure to simulated tidal turbine noise.

This project will assess the potential for hearing loss and tissue damage to ESA-listed juvenile Chinook salmon after exposure to a simulated tidal turbine noise. The results will enable Snohomish PUD and tidal turbine developers and regulatory authorities to better understand the potential for adverse impacts to an ESA-listed species and to work collaboratively to ensure pilot- and full-scale MHK development is protective of the aquatic environment.

2.0 General Methods and Results

2.1 Test Organism Collection, Handling, and Care

The collection, handling, and care of juvenile Chinook salmon were conducted in accordance with state and federal requirements. The experimental procedures for measuring hearing sensitivity were approved by the Pacific Northwest National Laboratory (PNNL) Animal Care and Use Committee.

The average fork length (FL) and weight of smolts used in this study (mean ± standard error of the mean [SEM]) were 125 ± 0.52 mm and 20.9 ± 0.263 g, respectively. Fish were supplied by Leavenworth National Fish Hatchery, Leavenworth, Washington, and transported to PNNL, Sequim, Washington, where they were held for the duration of the study (March 3, 2011–June 9, 2011) at an average temperature of 9.8 °C ± 0.006.

The salmon smolts were acclimated to ambient salt water levels (about 32 ppm) over 3 weeks. In order to expose the fish as a marine species, the fish were compelled into early smoltification during March; smolting would normally occur around May. There are no studies to determine if hearing or tissue sensitivities differ for fish when they are physiologically adapted to fresh water versus salt water. The early transition into salt water was to test fish that have migrated out into the coastal ocean and Puget Sound, where tidal turbines are proposed to be installed.

2.2 Treatment Paradigm

The juvenile Chinook salmon were maintained during the study in holding tank 6 (Ht-6). The volume of this tank was 2000 L. A subgroup of 75 fish were transferred out of Ht-6 and into treatment tank 1 (Tt-1) on the morning of the Friday preceding treatment. From Friday morning until Tuesday evening, these fish were not fed to decrease the chance of injury from hard pellets in their guts and to eliminate food in the gut as a study variable. The time period from Friday until Monday morning also allowed the fish to acclimate to the treatment tank environment and the presence of fewer conspecifics.

The fish were treated from 9 a.m. on Monday until 9 a.m. on Tuesday with 24 hours of tidal turbine sound exposure, or 24 hours of no sound (control). Sampling for each assessment, tissue injury and hearing, commenced immediately at the end of the treatment. After sampling was completed on Tuesday, all the treatment fish were transferred from Tt-1 into a 300-L holding tank Ht-216 where further post-treatment samples were taken on days 1, 3, and 6 (see Figure 2.1, a flow chart of the handling, treatment, and post-treatment assessment of test fish).

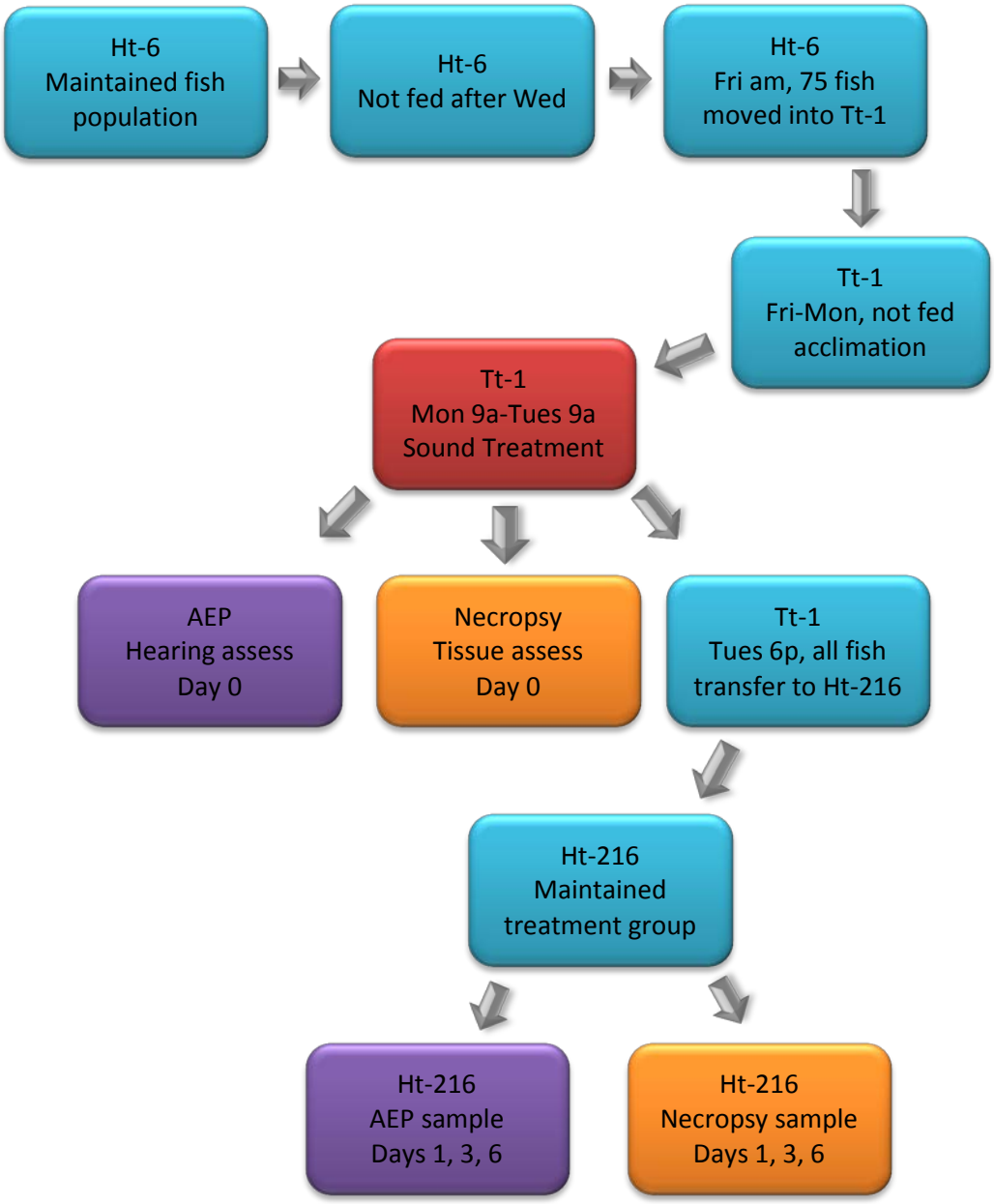


Figure 2.1. Treatment and Sampling Process

The treatment, assessment objective, and number of fish (*n*) processed are shown in Table 2.1. There were six treatment groups; four were sound exposed and two were control, no sound exposure. The scientists performing the assessment were unaware (blind) to the treatment the fish had received.

Table 2.1. Treatment Groups

Treatment	Assessment	Day Post-Treatment (*n*)			
		0	1	3	6
Treatment 1 - Exposure					
Exposure	Tissue dam	9	4	10	10
Baseline (neg control)	Tissue dam	4	5	5	5
Treatment 2 - Exposure	AEP	3	1	0	4
Exposure	Tissue dam	12	12	12	12
Baseline (neg control)	Tissue dam	3	5	5	4
Treatment 3 - Control	AEP	0	4	4	4
Control	Tissue dam	11	11	12	10
Baseline (neg control)	Tissue dam	4	5	5	4
Treatment 4 - Exposure	AEP	4	4	4	4
Exposure	Tissue dam	11	12	10	10
Baseline (neg control)	Tissue dam	5	5	4	5
Treatment 5 - Exposure	AEP	3	4	4	4
Exposure	Tissue dam	12	11	11	12
Baseline (neg control)	Tissue dam	5	4	4	4
Treatment 6 - Control	AEP	4	4	3	3
Control	Tissue dam	9	10	10	11
Baseline (neg control)	Tissue dam	5	4	3	3

2.3 Noise Exposure System

The noise exposure test tank, Tt-1, was a 5-mm-thick aluminum round tank, 91 cm in diameter × 76 cm high with a volume of 500 L (Figure 2.2). The tank was lined with a blue anechoic material (Aptflex F48 by Precision Acoustics LTD, Dorchester, United Kingdom) 2.6 cm thick and a density of 1.91 gm/ml to stiffen the walls and create a uniform sound field. Vibration isolation feet were attached to each tank leg to decrease low-frequency vibrations coming into the tank and reduce the transfer of simulated turbine noise into the laboratory through the tank's contact with the floor. A UW30 speaker (Lubell Labs Inc., Columbus, Ohio) was placed in the bottom center of the tank, connected to a Hafler P1000 preamplifier (Rockford Corp, Tempe, Arizona) and driven by a Sony PCM-D50 Recorder, which played back a recording of simulated tidal turbine noise.

A Reson TC4013 hydrophone (Reson Inc., USA; sensitivity −211dB re 1V/µPa) was hung off of a plastic tube into the center of the water in the tank at a depth of approximately 30 cm. This placement of the hydrophone permitted measurement of the noise to which test fish were exposed. The test fish typically swam at this level in the tank over the period of sound exposure. The hydrophone output was connected to a B&K Nexus 2690A-OS4 conditioning amplifier (Bruel & Kjaer, Naerum, Denmark), then to a Measurement Computing USB-1608HS DAQ module (Measurement Computing, Norton, Massachusetts). The digitally sampled noise exposure waveform was written to storage using a Panasonic CF-29 Toughbook. System calibrations were performed using a B&K Type 4229 Pistonphone Calibrator.

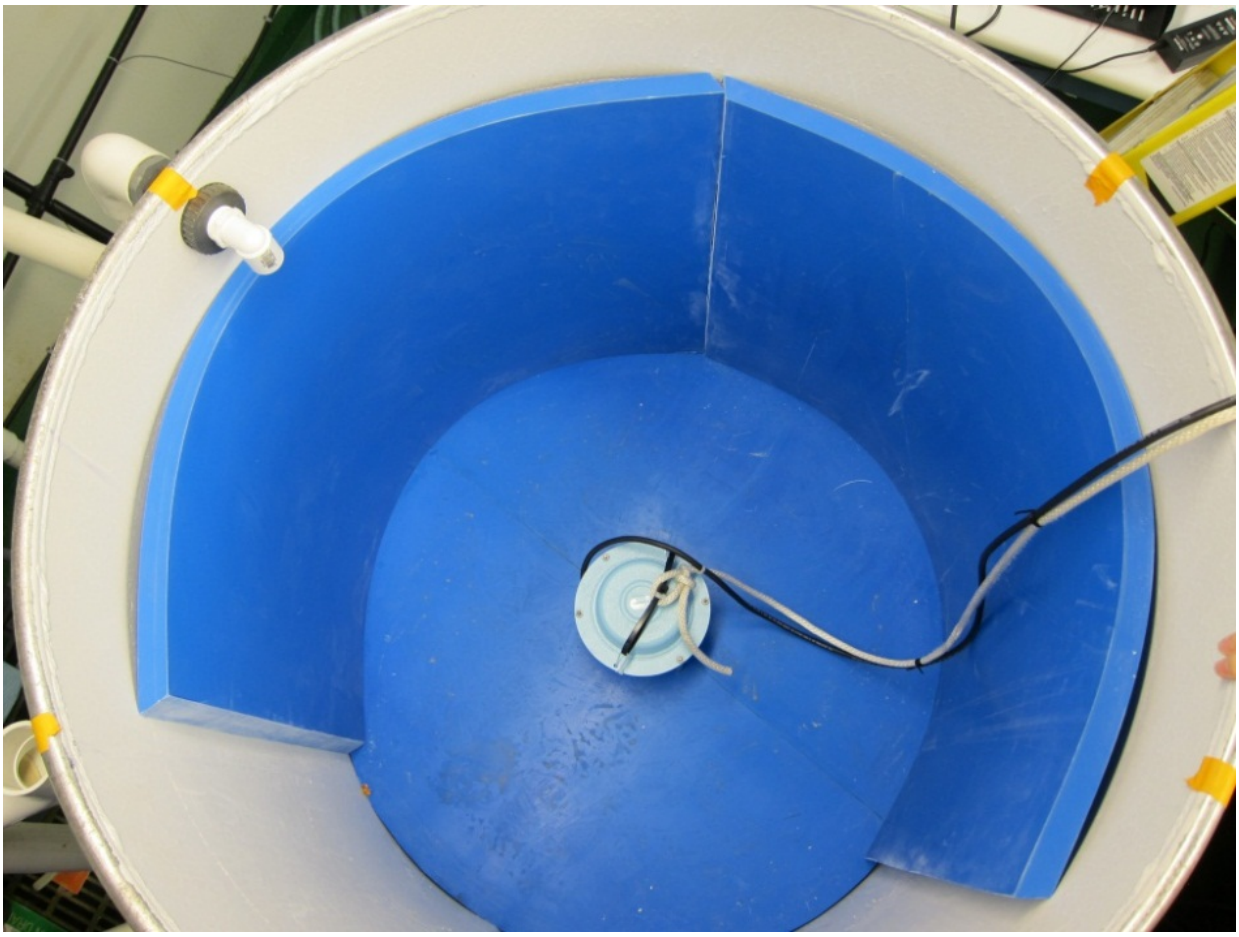


Figure 2.2. Sound Exposure Tank, Partial Assemblage

3.0 Tidal Turbine Noise

3.1 Noise Generation

A sample of the spectra of sound generated by an operating 6-m OpenHydro turbine being tested at the European Marine Energy Center was provided by project partners at the University of Washington (Polagye et al., 2011); Figure 3.1). These spectra were used to develop a time domain waveform that simulated the noise generated by an OpenHydro tidal turbine of the size to be deployed in Admiralty Inlet. The frequency band of interest was 100 to 400 Hz.

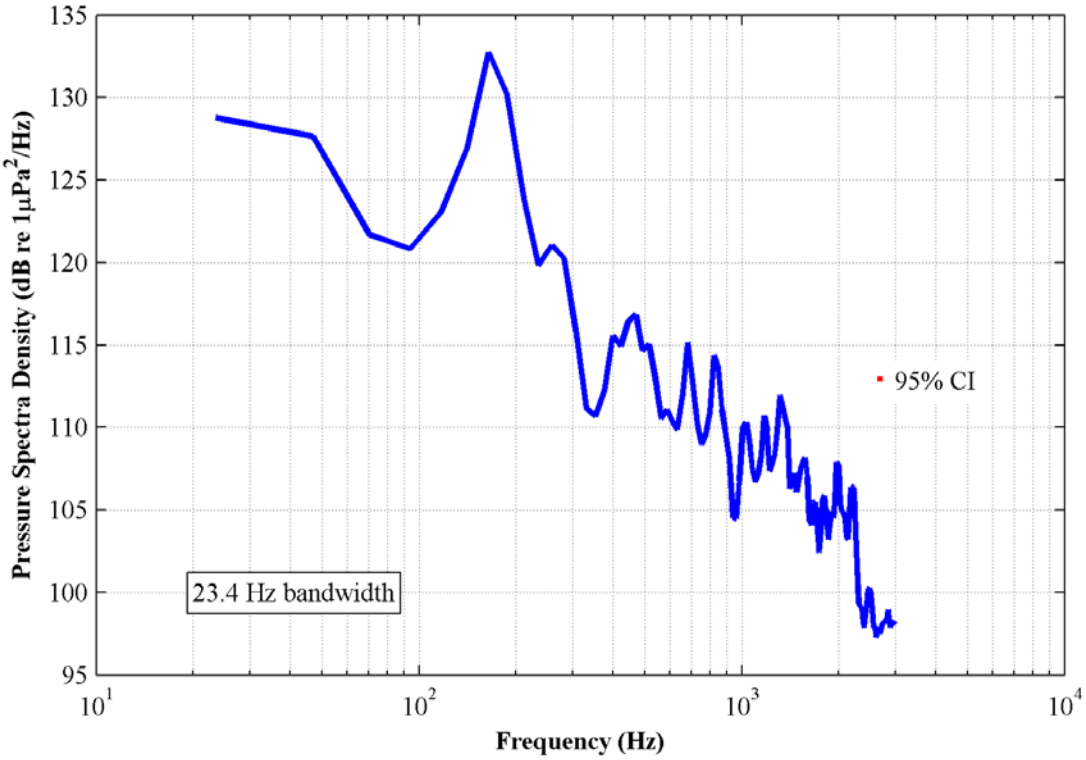


Figure 3.1. Recorded Spectra of Sound Generated by a 6-m-diameter OpenHydro Tidal Turbine

3.2 Noise Recordings and Statistics

The sound levels for the treatment groups (SPL_{rms}) ranged from 155 to 163 dB re 1 µPa rms (Table 3.2 and Figure 3.2). Treatment 1 in Figure 3.2 has a region that became flattened out. We are confident this was an issue only with the recording equipment because the output voltage signal to the underwater speaker was monitored and was within the normal range during the entire exposure duration. The treatment controls were not exposed to simulated tidal turbine noise, as shown by the black trace in Figure 3.2.

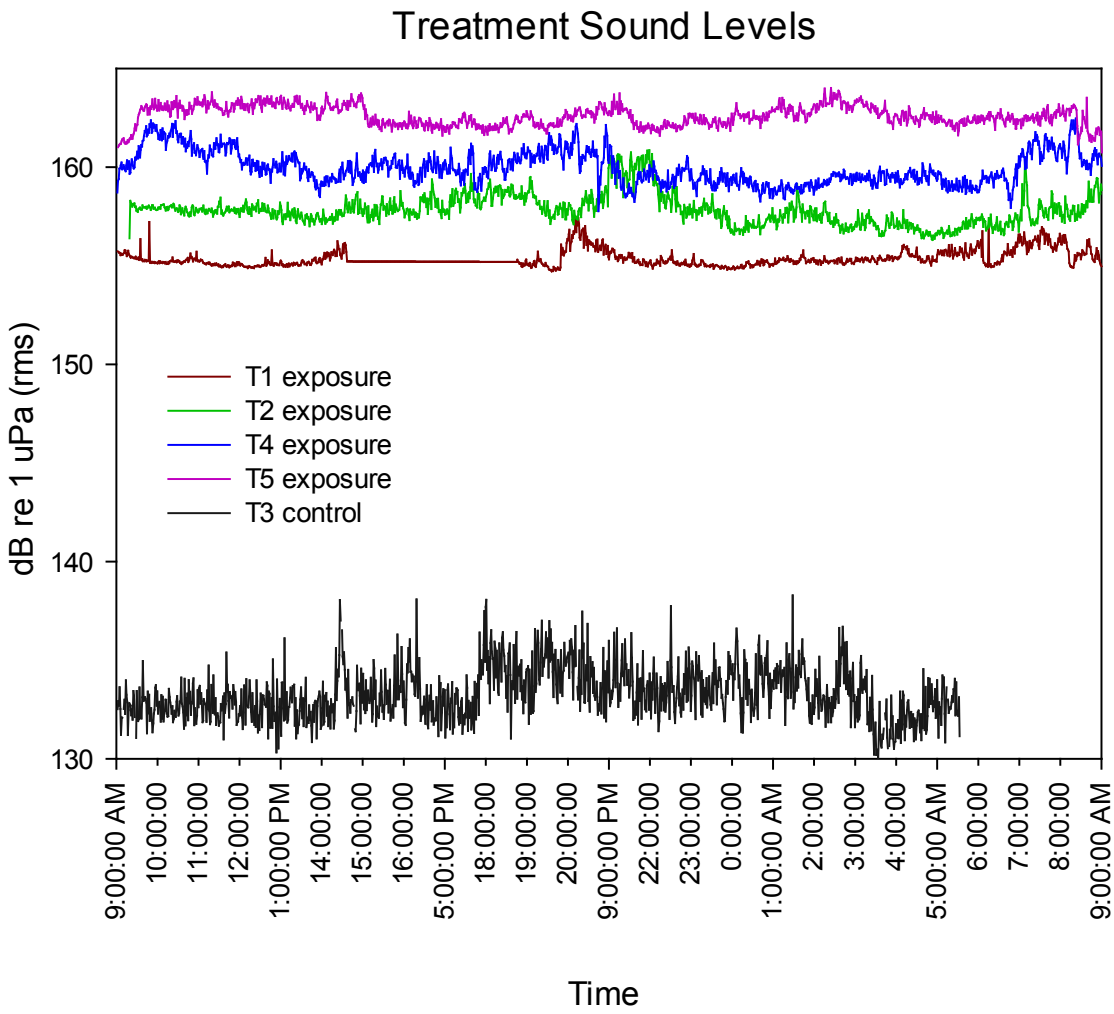


Figure 3.2. Treatment Sound Levels

3.3 Exposure Noise Statistics and Results

Each treatment exposure or treatment control was analyzed using one-way analysis of variance (ANOVA) to compare exposure levels and post hoc using Tukey analysis. Statistical analysis also included descriptive statistics and the ANOVA were performed using MiniTab 16 (Minitab, Inc., State College, Pennsylvania).

The treatment exposures were significantly different ($p < 0.0001$) (Table 3.1), and post hoc analysis shows that each was significantly different by the groupings value in Table 3.2. A 6-dB level difference in rms sound pressure level (SPL_{rms}) is a doubling (or halving) of exposure level. The difference in level of noise between treatments was not uniform; therefore, all the treatments were analyzed separately.

Treatments 3 and 6 were the control exposures, and the sound recording equipment was not turned on for Treatment 6; therefore, there were no data to compare the sound level in the tank between the two control groups. However, because no turbine sound was turned on and there were no other changes in the

laboratory environment when the control groups were being held, there is no reason to expect that the sound level in the control tank would have differed between the two time periods. Treatment control 3 was statistically different from all treatment exposures (Table 3.2).

Table 3.1. Noise ANOVA

Source	DF	SS	MS	F	P
Factor	4	706446.0	176611.5	265678.17	< 0.0001
Error	6982	4641.3	0.7		
Total	6986	711087.4			

Table 3.2. Noise Post Hoc Tukey Analysis Results

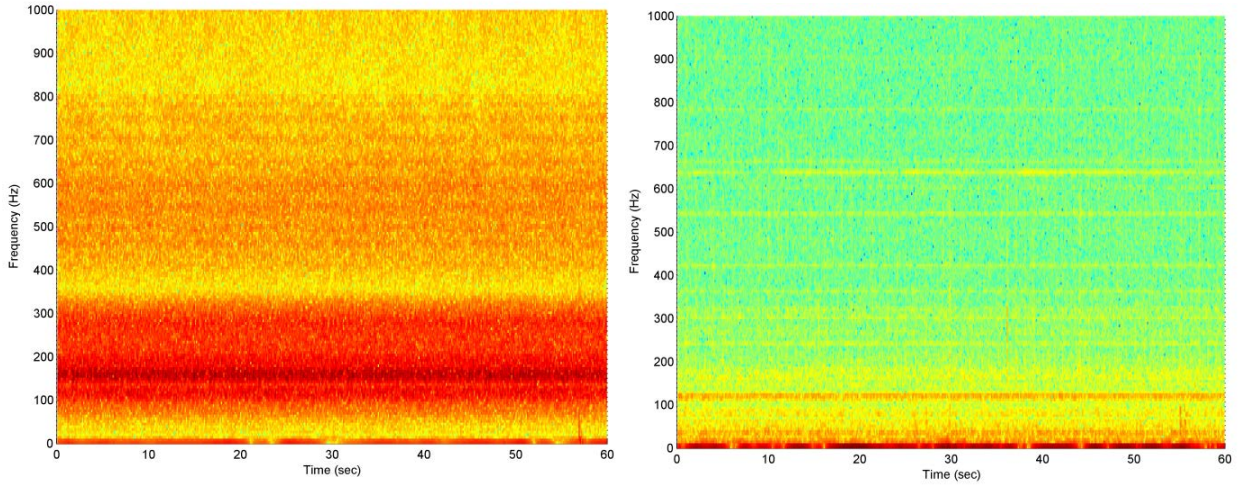
Level	N	Mean SPL_{rms} dB re 1µPa	StDev	Grouping
T1 exposure	1439	155.358	0.406	A
T2 exposure	1441	157.821	0.786	B
T4 exposure	1444	159.932	0.843	C
T5 exposure	1439	162.557	0.514	D
T3 Control	1224	133.235	1.313	E

Grouping information using Tukey method.
Means that do not share a letter are significantly different.

An example of a treatment exposure signal and the treatment control are shown in Figure 3.3. The darker colors in the spectrogram (top row) identify frequencies of higher energy relative to the light color, which indicate lower sound energy levels at specific frequencies as they change with time over the duration of the exposure signal. The power spectral density (PSD) plots in the bottom row show similar information, but these plots include the entire simulated tidal turbine sound sample, which means more information is gathered into the PSDs than in the respective spectrograms.

A. Treatment 4, Spectrogram, 1 min

C. Treatment Control, Spectrogram, 1 min



B. Treatment 4, Power Spectral Density

D. Treatment Control, Power Spectral Density

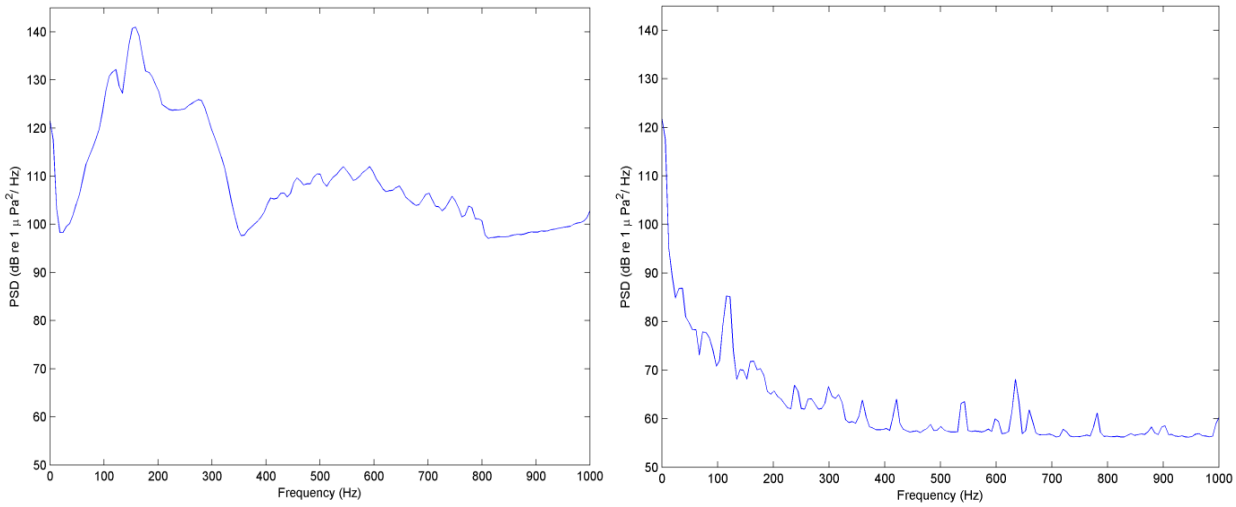


Figure 3.3. Spectrogram and Power Spectral Density for Exposure Signal (A/B) and Control (C/D)

4.0 Auditory Assessment

Fish ears function as inertial accelerometers (Fay, 1984), but some fish have specialized auditory structures for enhanced pressure reception. Chinook salmon are in the salmonid family, and they lack auditory specializations for enhanced pressure sensitivity. After 24 hours of exposure, samples of exposed fish were processed to assess their hearing and occurrence of tissue damage. Fish not immediately processed were placed in holding tank Ht-216 for later examination (Table 2.1).

4.1 Auditory Evoked Potential Methods

Assessment of the auditory system uses auditory evoked potential (AEP), a hearing assessment technique that is noninvasive and relatively quick to conduct (e.g., (Corwin et al., 1982; Kenyon et al., 1998; Halvorsen et al., 2009). A total of 80 juvenile Chinook salmon were individually placed into a 33.6-cm-ID × 35.5-cm-high steel cylinder with a 0.95-cm wall thickness supported by three vibration-dampened legs, filled to a depth of 30.5 cm with salt water. An underwater speaker (Model UW30, Lubell Labs, Columbus, Ohio) was mounted through the bottom of the tank. Fish were injected intramuscularly, just below the dorsal fin, with a neuromuscular block called Flaxedil (gallamine triethiodide, SIGMA ALDRICH, St.Louis, MO) at an approximate dose of 0.0003 mg/g. The animal was suspended 12.5 cm below the water surface in a soft cloth sling inside of the hearing test tank, keeping the head and opercula clear of obstruction. A tube was placed into the fish's mouth, which had gravity-fed water flow to force water over the gills and allowed for continuous respiration of the animal (Figure 4.1).

Electrodes (Rochester Electro-Medical, Tampa, Florida) were insulated with nail polish except for leaving 2 mm of the tip exposed and sharp enough to be directly inserted to a 2-mm depth under the skin. One electrode was placed subcutaneously between the nares—this was the reference electrode. The second electrode was placed on the dorsal surface and just posterior to the cranium (top of the head)—this is called the recording electrode. A grounding electrode was placed in the water (Figure 4.2A). When a tone is played in the water, the fish ears detect the sound and the electrode can pick up the brain's synchronized neural response (Figure 4.2B). The brain response is digitally stored and processed at the time of testing. During each recording session, a Reson TC4013 hydrophone was affixed lateral to the fish to record and analyze the received acoustic stimulus. Once an animal completed an AEP test, it was not retested.

Stimulus generation and AEP collection were done using Tucker-Davis-Technologies equipment (TDT, Alachua, Florida). The stimulus signals were software-generated in SigGen (TDT) and used in BioSig (TDT) (Figure 4.2B). They were played out through a TDT System 3 (RP2.1) real-time signal processing module and passed through a power amplifier (Hafler P1000, Columbus, Ohio) connected to the underwater speaker (UW30). The presented test signals were 100-, 200-, 300-, and 400-Hz tones. All tones had 3-ms Hanning rise and fall times, and the signal duration was 59 ms for 100 and 200, 300, and 400 Hz, presented at a rate of 15.38 per sec using a window length of 65.0 ms. The acoustic stimuli were monitored with a Reson TC4013 hydrophone (sensitivity of −212.5 dB re 1 V/µPa) (RESON A/S, Slangerup, Denmark) connected to a Kistler 5010 dual-mode amplifier (Kistler Instrument Corp., Amherst, New York), into the RP2.1 and connected to a laptop computer.

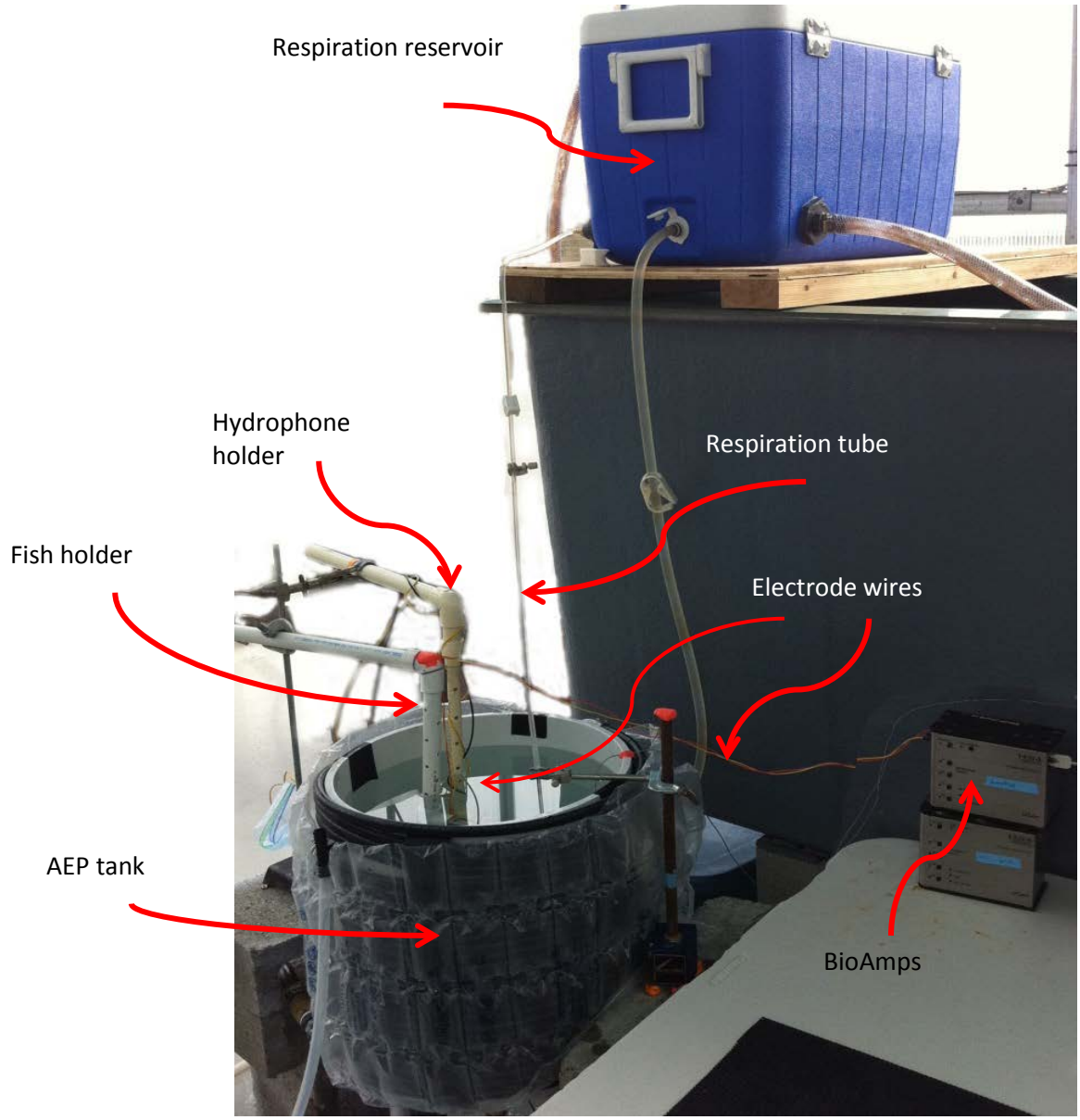
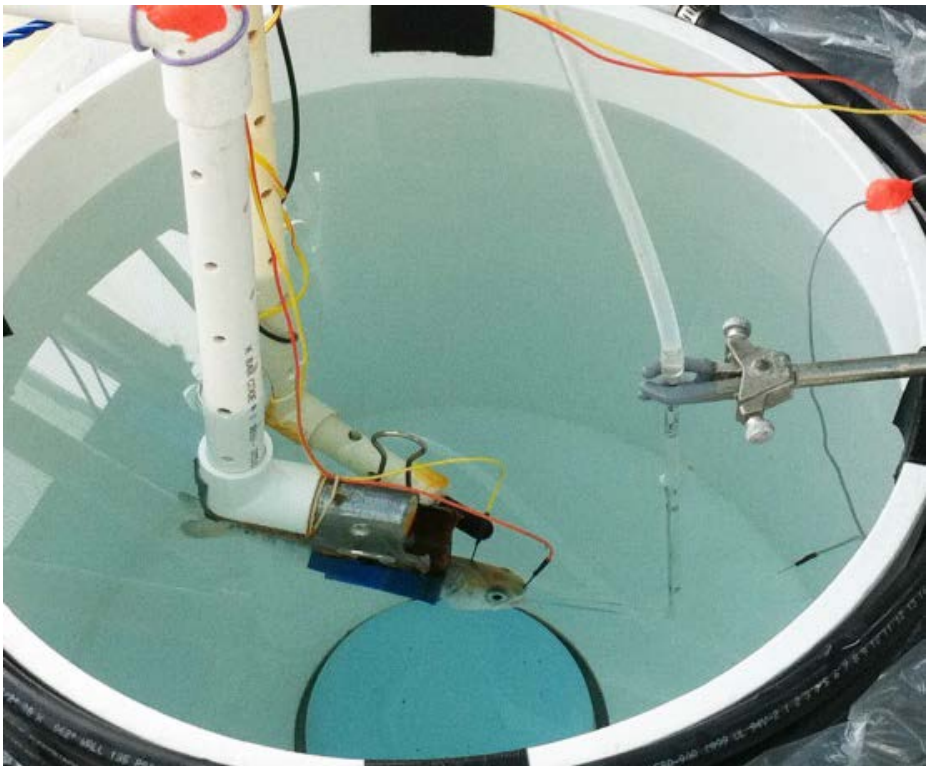


Figure 4.1. AEP Setup

The recording equipment consisted of the RP2.1 module with an HS4 head stage (TDT) and a DB4 filtering attenuator (TDT). AEP traces were band-pass filtered from 30 Hz to 3,000 Hz, then sent to TDT RP2.1 and digitized at 25 kHz. All AEP acquisition was done using the BioSig software package (TDT) with 500 responses averaged for each presentation (i.e., 500 stimuli of 0° polarity and 500 stimuli of 180° polarity). The alternating phase was to cancel out electrical artifacts on the AEP electrodes. Water was changed between fish, and the temperature was recorded at the beginning and end of each trial.

To determine hearing thresholds, the stimulus (sound pressure) level was decreased in 6-dB increments until an AEP waveform was no longer visually distinguishable from the background noise. Threshold was defined as the lowest level in which an AEP response was recorded. The traditional determination of threshold using visual inspection provides results that are similar to those determined using statistical approaches (Mann et al., 2001; Brittan-Powell et al., 2002).

A.



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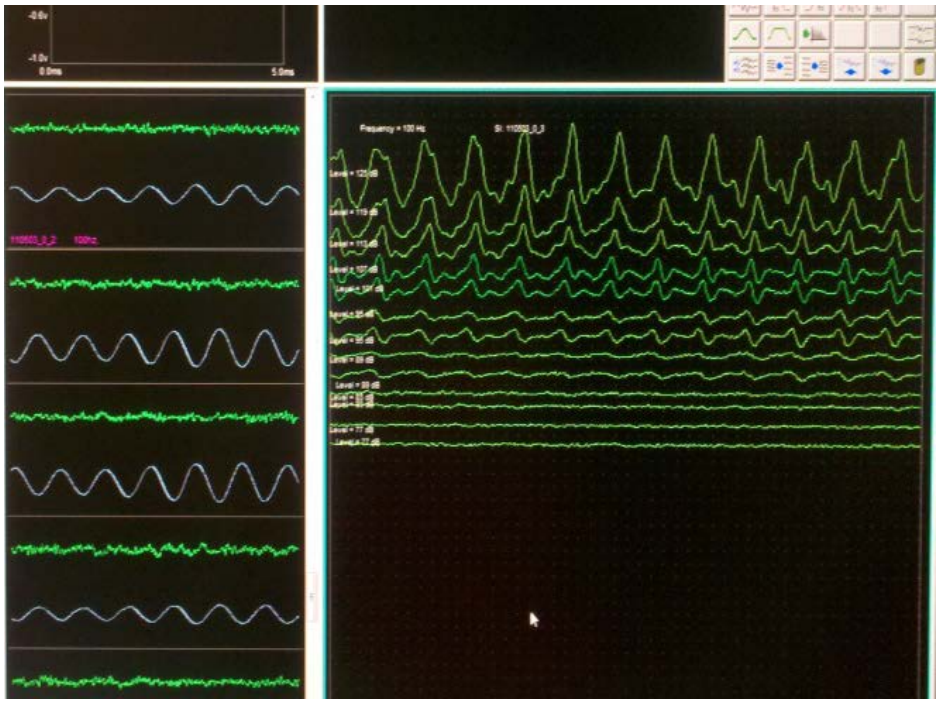

Figure 4.2. Fish Undergoing an AEP Hearing Test (A); Electrophysiological Response (B)

4.2 Auditory Statistical Methods

The treatment groups were defined by necropsy time after the treatment (i.e., 0, 1, 3, or 6 days). One-way ANOVA was used to compare threshold values, followed by Tukey post hoc analysis. Statistical analysis also included descriptive statistics and the ANOVA and Tukey post hoc were performed using MiniTab 16 (Minitab, Inc., State College, Pennsylvania).

4.3 Auditory Results

AEP traces of the brain response from a 100-Hz tone stimulation in a juvenile Chinook salmon are shown in Figure 4.3.

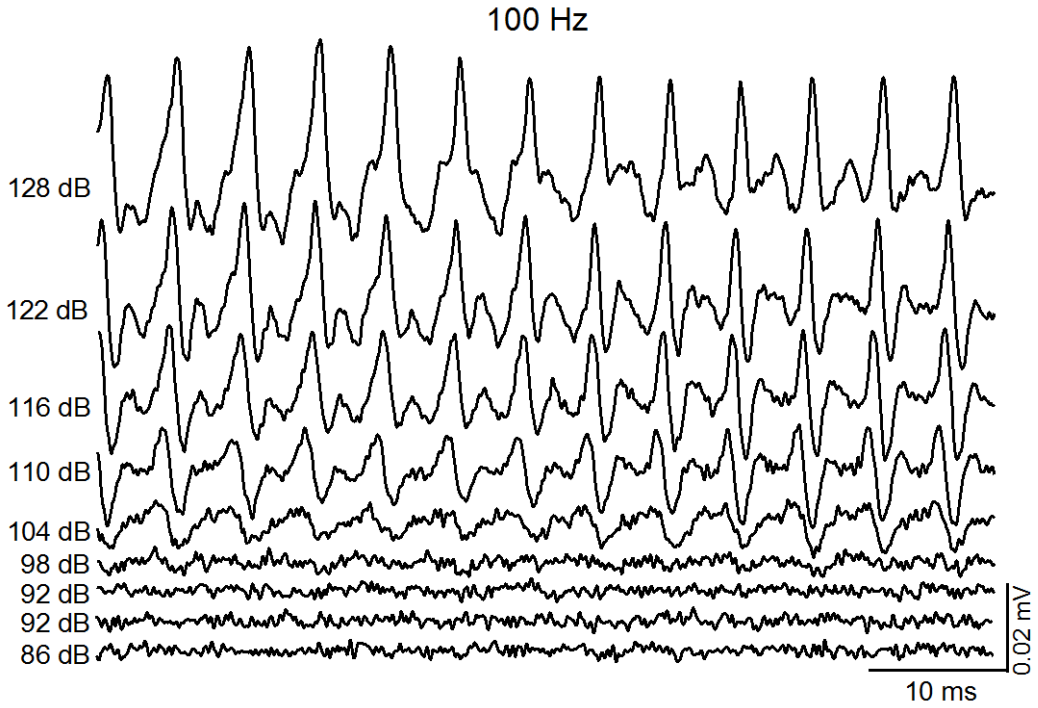

Figure 4.3. Evoked Potential Traces from 100-Hz Tone Stimulus.

ANOVA comparisons of all treatments * frequencies did not show a significant difference of p = 0.326 (Table 4.1). The Tukey post hoc test showed significant differences between days 3 and 6 compared to control days 3 and 6, respectively, while days 0 and 1 showed no significant differences (Table 4.2). However, fish in treatment days 3 and 6 (Figure 4.4) have better hearing than those in treatment controls, and the shapes of the audiograms were a bit unusual in that they did not follow the J-shaped curve evident in day 0 treatments (Figure 4.4). AEP analysis to date indicates that the sound exposure did not have a definitive negative effect on test fish.

Table 4.1. AEP ANOVA Results

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatment	7	1603.17	1504.62	214.95	4.33	< 0.0001
Frequency	3	5155.40	5003.51	1667.84	33.63	< 0.0001
Treatment*freq	21	1169.31	1169.31	55.68	1.12	0.326
Error	210	10416.16	10416.16	49.60		
Total	**241**	**18344.04**				

Table 4.2. AEP Tukey Post Hoc Results

Treatment	N	Mean SPL_{rms} dB re 1 µPa	Grouping
Ctrl 0	16	91.6	A B
PE 0	40	95.3	A B
Ctrl 1	28	95.0	A B
PE 1	35	91.2	A B
Ctrl 3	27	96.2	A
PE 3	32	90.2	B
Ctrl 6	32	96.1	A
PE 6	32	90.3	B

Grouping Information Using Tukey Method. Means that do not share a letter are significantly different, only pairs with the same time point (day 0, 1, 3, 6) need to be compared.

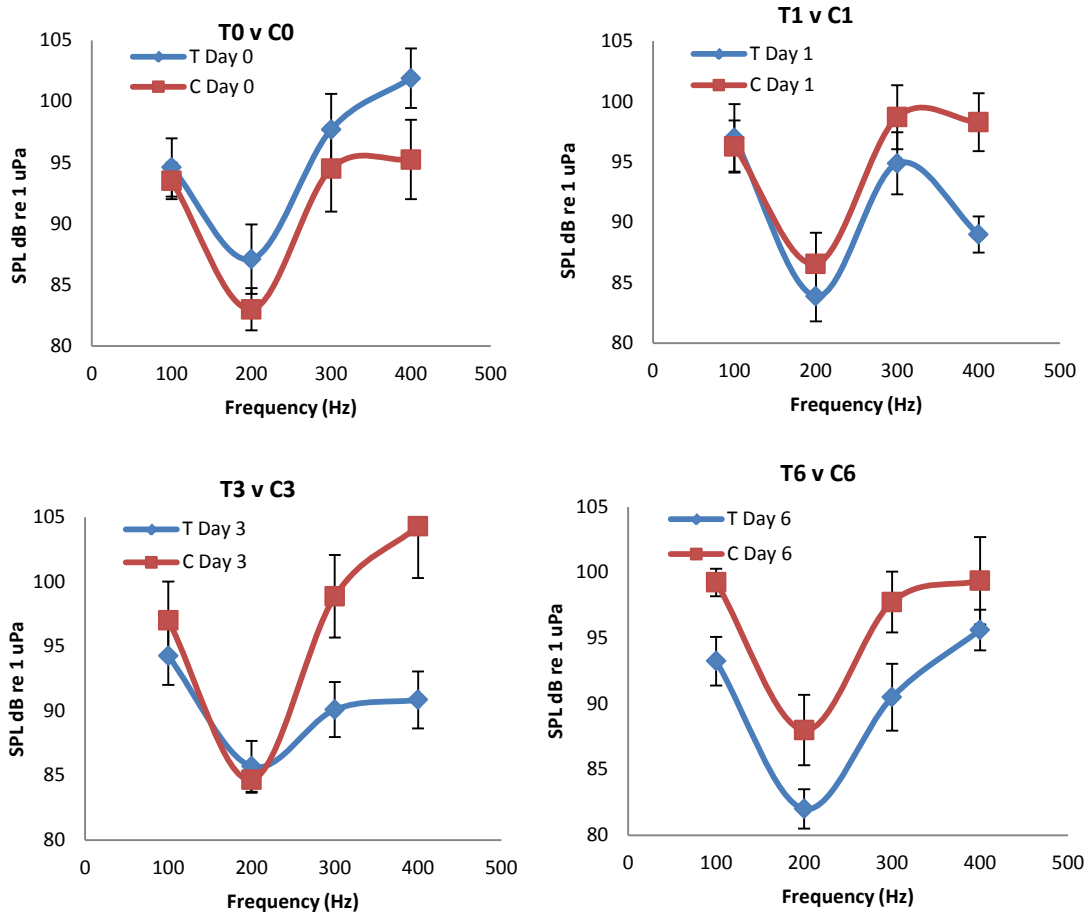

Figure 4.4. Audiogram Curves for Each Day Post-Exposure. The relative hearing sensitivity is represented by the y-axis, the frequency range in on the x-axis. Controls are designated with a red box and treatments with a blue diamond.

5.0 Tissue Damage

Chinook salmon are a physostomous fish, which means they must volitionally gulp air at the surface to fill their swim bladder to attain neutral buoyancy. They are also capable of volitionally releasing air from their swim bladder to manage their buoyancy. The response of fish to sound exposure requires that their physiological state be known and be uniform across the samples of treatment and control fish. It is important that researchers provide test fish the opportunity to attain neutral buoyancy prior to exposure, which was satisfied for this study with 3 days of acclimation in tank Tt-1 with access to free air at the water surface. Studies have shown that negatively buoyant fish (a deflated swim bladder) are protected from barotrauma from exposure to rapid decompression (Stephenson et al., 2010) and also from pressure changes caused by exposure to sound (Michele Halvorsen, PNNL, personal observation).

5.1 Tissue Damage Methods

Salmon were examined externally and by necropsy for internal inspection to determine if physical injuries resulted from exposure to simulated tidal turbine sound(n = 381) at four different times following exposure: day 0 – exam immediately after exposure; day 1 – one day after exposure; day 3 – three days after exposure; day 6 – six days after exposure. Prior to examination, fish were euthanized in a temperature-controlled and buffered solution of 250 mg of tricaine methanesulfonate (MS-222)/L of water. Each fish was inspected for external injury and then inspected for internal tissue damage by necropsy. Injury assessment was guided by reference to a panel of 72 potential tissue injuries developed over several years of investigation of the response of fish to rapid decompression and exposure to sound. The scientists were blind to the fish's exposure treatment. The methodology for the assessment of tissue damage followed the procedures developed and refined by Halvorsen et al. (2011) and Carlson et al. (2011). Those studies developed a method for quantitative assessment of the physiological cost of barotrauma injuries to fish exposed to rapid decompression and changes in pressure resulting from exposure to sound. Table 5.1 presents examples of external and internal injuries that can be sustained from sound exposure.

5.2 Fish Index of Trauma

Tissue damage injuries can range from non-lethal to lethal, depending on response to exposure to sound. Non-lethal injuries include effects such as scale loss, impact to sensory systems, and/or changes in behaviors that increase the risk of exposure to predation by piscivorous fish, marine mammals, and birds (Popper et al., 2004; Schreer et al., 2009). Lethal injuries include tissue laceration, embolisms, hemorrhage, and other injuries that severely compromise the physiology of the exposed fish.

The physiological cost of many sublethal injuries are poorly understood in fish; thus, a novel model was developed to qualitatively assess barotrauma across the range of injury from mild to mortal. This method was in two other studies involving impulsive sound and explosive sound effects on fish (Carlson et al. 2011; Halvorsen et al. in press). The physiological significance of each injury was determined using available literature (Husum et al., 2002; Oyetunji et al., 2010) ,and proposed energetic costs were based on understanding each injury type (Michele Halvorsen and Christa Woodley, PNNL, personal observations).

Table 5.1. Abbreviated List of Tissue Injuries

External Injuries	Internal Injuries
Dead or Moribund	Distended Swim Bladder
Damage: Eye(s)	Internal Enlarged: Internal Capillaries/Vessels
Emesis	Internal Hematoma On Body
Scale Loss	Hematoma: Gall Bladder (Pink Or Red)
Exophthalmia: Eye(s)	Hematoma: GI Tract
External Hematoma on Body	Hematoma: Hepatic
Hematoma: Anal Fin	Hematoma: Ovaries/Testes
Hematoma: Caudal Fin	Hematoma: Pericardial
Hematoma: Dorsal Fin	Hematoma: Renal
Hematoma: Pectoral Fin	Hematoma: Swim Bladder
Hematoma: Pelvic Fin	Hematoma: Vent (Blood Spots)
External Hemorrhage on Body	Hematoma: Fat
Hemorrhage: Anal Fin	Deflated: Swim Bladder (No Ruptures)
Hemorrhage: Caudal Fin	Hemorrhage: Capillaries
Hemorrhage: Dorsal Fin	Hemorrhage: Fat
Hemorrhage: Eye(s)	Hemorrhage: GI Tract
Hemorrhage: Gill(s)	Hemorrhage: Liver
	Hemorrhage: Pectoral Fin
	Hemorrhage: Pelvic Fin
	Hemorrhage: Pericardial
	Hemorrhage: Pyloric Caeca
	Hemorrhage: Renal
	Hemorrhage: Spleen
	Hemorrhage: Swim Bladder
	Damage: Tear, Laceration

Examination of the injury panel showed that not all injuries had the same physiological significance for the health of the fish following exposure. Therefore, the classification system, which includes consideration of injury severity in addition to the presence of injury, was applied to the observed injuries, and three injury classes were used—mortal, moderate, and mild. The level of tissue damage for each injury in the assessment panel was based on scores (0 – no injury present; 1 – minor injury; 2 – moderate injury; 3 – severe injury) that denote the severity of observed external and internal injuries.

Each injury class was weighted on the basis of its physiological costs. Mild injuries, weighted with 1, potentially increase energetic costs to the fish, although they are unlikely to affect their overall baseline performance. Moderate injuries, weighted with 3, include those that are physiologically costly. The fish would be likely to recover from the injury; however, the baseline performance would be affected. Furthermore, under additional stress, the fish may suffer prolonged recoveries, delayed mortality, or increased predation. Mortal injuries, weighted with 5, tend to be life threatening with immediate or delayed mortality risks.

The product of the severity score and the weighting for each injury provided a Response Severity Index for each fish:

$$RSI = \sum (Severity * Weight)$$

One of the strengths of this model is the incorporation of the severity of the observed physical injury and the physiological cost of the observed injury. The scores given to severity (0 to 3) and physiological cost (1, 3, or 5) calculate to yield a final score, RSI. The RSI was calculated for each fish, including control treatments.

5.3 Tissue Damage Statistical Methods

The biological response was reported in RSI, and each treatment group had to be analyzed independently because each of the treatment signal levels was statistically different. Each treatment was further defined by necropsy time after the treatment (i.e., 0, 1, 3, or 6 days). One-way ANOVA was used to compare RSI values for each day within a treatment, followed by Tukey post hoc analysis. Statistical analysis included descriptive statistics, ANOVA, and the Tukey post hoc were performed using MiniTab 16 (Minitab, Inc., State College, Pennsylvania).

5.4 Tissue Damage Results

After 24 hours of exposure to tidal turbine-like noise, fish were necropsied. Their tissues were examined for injury, and those results were plugged into the RSI model. Figure 5.1 shows the response severity index for each fish across all of the treatments, providing a graphical overview of all the tissue damage data. The comparisons between treatment and control are shown for each treatment test to show the results in detail.

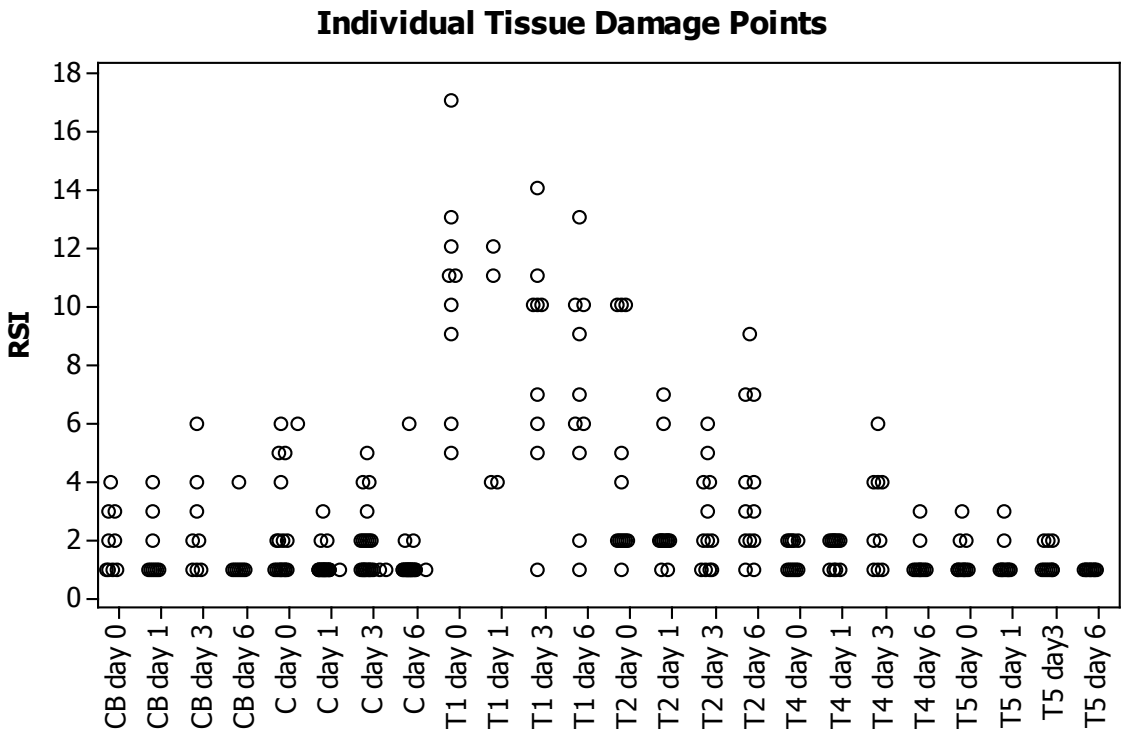

Figure 5.1. Tissue Damage, Control vs. All Treatments. CB = control baseline, these are the negative controls for the control fish, C = control, T1 = treatment 1, etc. See Figure 3.2 for details on treatment exposure levels. Each open circle represents an RSI value for an individual fish.

An ANOVA comparison between the baseline fish and treatment controls was significant ($F_{7,114}$ = 2.11; p = 0.05), suggesting that handling effects appeared in the controls, however Tukey post hoc analysis showed no statistical differences. Therefore the treatment control RSI values were similar to baseline RSI values (Figure 5.1) A comparison between treatment controls and Treatment 1 showed a significant difference (Table 5.2) at days 0, 1, 3, and 6 respectively (Table 5.3) and Figure 5.2.

Table 5.2. Tissue Damage ANOVA Treatment 1

Source	DF	SS	MS	F	P
Factor	7	1140.12	162.87	32.85	< 0.0001
Error	107	530.54	4.96		
Total	**114**	**1670.66**			

Table 5.3. Tissue Damage Tukey Post Hoc Treatment 1

Treatment	N	Mean RSI	Grouping
C day 0	18	2.389	C
T1 day 0	9	10.444	A
C day 1	20	1.200	C
T1 day 1	5	8.000	A B
C day 3	22	1.818	C
T1 day 3	10	8.000	A B
C day 6	21	1.333	C
T1 day 6	10	6.900	B

Grouping information using Tukey method. Means that do not share a letter are significantly different, only pairs with the same time point (day 0, 1, 3, 6) need to be compared.

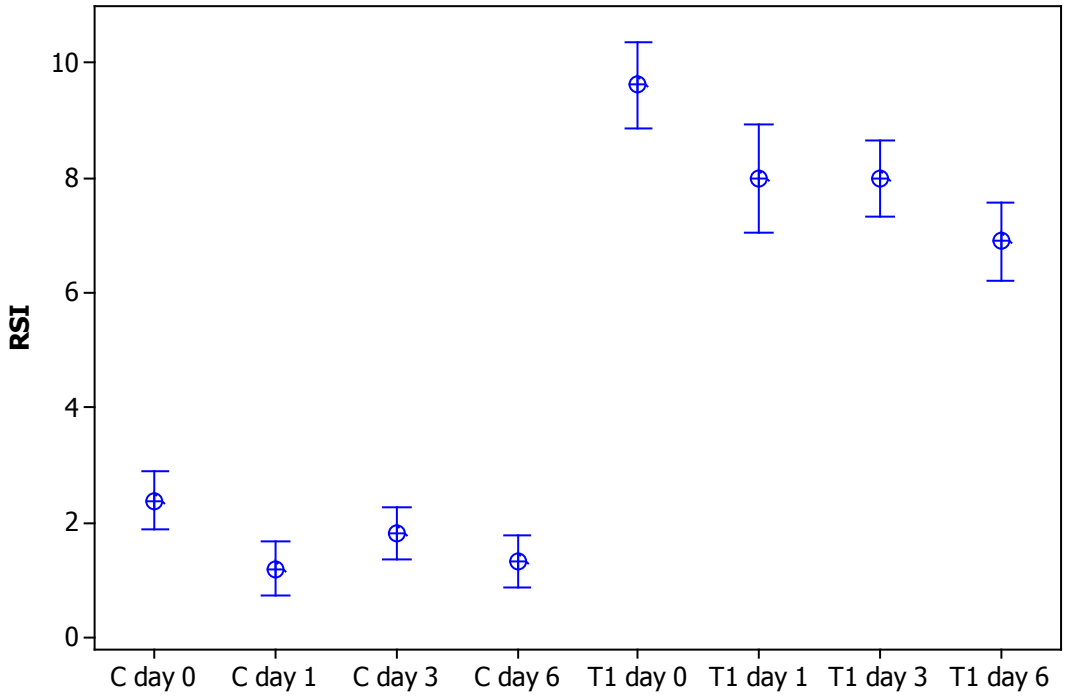

Figure 5.2. Tissue Damage, Control vs. Treatment 1. The open circles represent the average for each sample group with the standard error of the mean bars. The *y*-axis is the RSI values; the *x*-axis is the treatment groups, C and T1, separated by sample day (0, 1, 3, 6). All of the Treatment 1 time points show a significant level of injury.

The ANOVA comparison between the treatment controls and Treatment 2 showed a significant difference (Table 5.4). However, upon inspection of the post hoc analysis, there was one difference between treatment controls and Treatment 2 at day 6 (Table 5.5, Figure 5.3).

Table 5.4. Tissue Damage ANOVA Treatment 2

Source	DF	SS	MS	F	P1
Factor	7	126.47	18.07	5.39	< 0.0001
Error	121	405.92	3.35		
Total	**128**	**532.39**			

Table 5.5. Tissue Damage Tukey Post Hoc Treatment 2

Treatment	N	Mean RSI	Grouping
C day 0	18	2.389	A B C
T2 day 0	12	4.333	A
C day 1	20	1.200	C
T2 day 1	12	2.583	A B C
C day 3	22	1.818	B C
T2 day 3	12	2.667	A B C
C day 6	21	1.333	C
T2 day 6	12	3.750	A B

Grouping Information Using Tukey Method.
Means that do not share a letter are significantly different, only pairs with the same time point (day 0, 1, 3, 6) need to be compared.

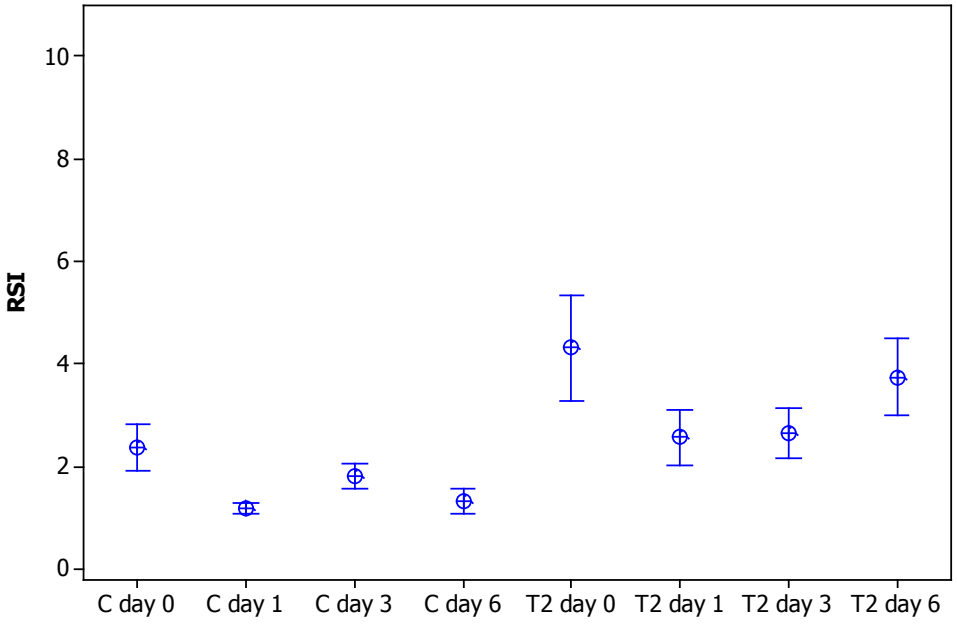

Figure 5.3. Tissue Damage, Control vs. Treatment 2. The open circles represent the average for each sample group with the standard error of the mean bars. The *y*-axis is the RSI values; the

x-axis is the treatment groups, C and T2, separated by sample day (0, 1, 3, 6). For all of Treatment 2 samples, there is a decrease in the level of RSI compared with Treatment 1.

The ANOVA comparison between the treatment controls and Treatment 4 showed a significant difference (Table 5.6); However, the post hoc analysis showed, no difference between treatment controls and Treatment 4 at days 0, 1, 3, and 6 respectively (Table 5.7, Figure 5.4).

Table 5.6. Tissue Damage ANOVA Treatment 4

Source	DF	SS	MS	F	P
Factor	7	37.65	5.38	2.57	0.017
Error	116	242.31	2.09		
Total	**123**	**279.96**			

Table 5.7. Tissue Damage Tukey Post Hoc Treatment 4

Treatment	N	Mean RSI	Grouping
C day 0	18	2.389	A B
T4 day 0	12	2.250	A B
C day 1	20	1.200	B
T4 day 1	11	1.636	A B
C day 3	22	1.818	A B
T4 day 3	10	3.000	A
C day 6	21	1.333	A B
T4 day 6	10	1.300	A B

Grouping information using Tukey method. Means that do not share a letter are significantly different, only pairs with the same time point (day 0, 1, 3, 6) need to be compared.

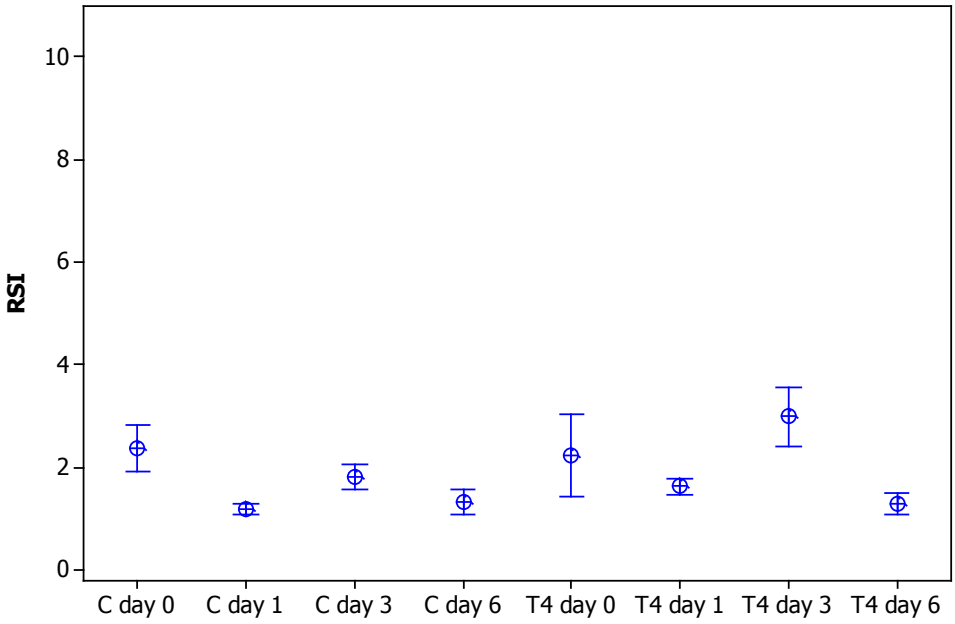

Figure 5.4. Tissue Damage, Control vs. Treatment 4. The open circles represent the average for each sample group with the standard error of the mean bars. The *y*-axis is the RSI values; the *x*-axis is the treatment groups, C and T4, separated by sample day (0, 1, 3, 6). For all of Treatment 4 samples, the level of RSI decreases in comparison to Treatment 1.)

The ANOVA comparison between the treatment controls and Treatment 5 did not show a significant difference (Table 5.8, Figure 5.5).

Table 5.8. Tissue Damage ANOVA Treatment 5

Source	DF	SS	MS	F	P
Factor	7	19.40	2.77	0.93	0.488
Error	123	367.72	2.99		
Total	**130**	**387.13**			

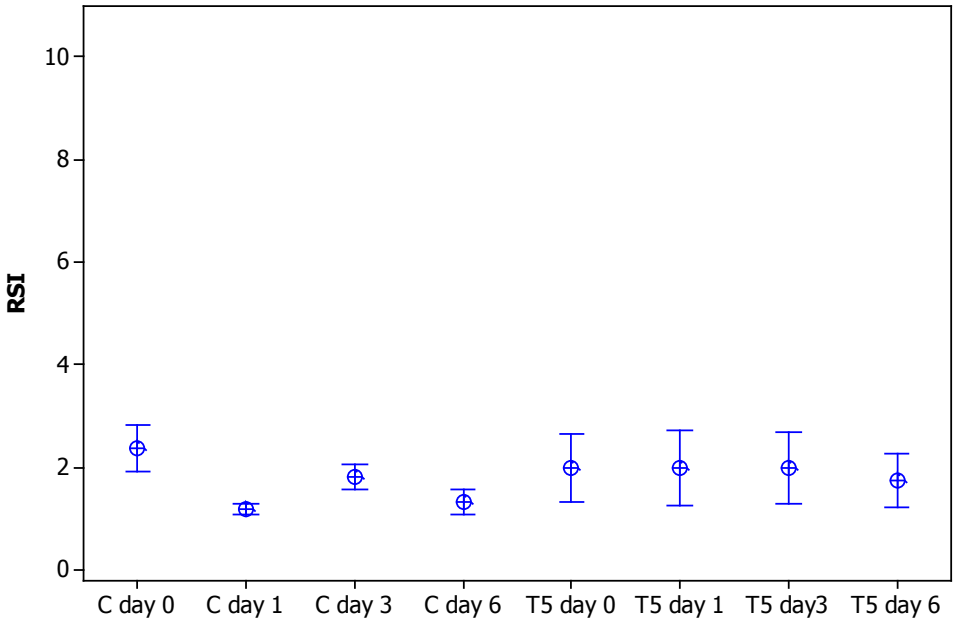

Figure 5.5. Tissue Damage, Control vs. Treatment 5. The open circles represent the average for each sample group with the standard error of the mean bars. The *y*-axis is the RSI values; the *x*-axis is the treatment groups, C and T5, separated by sample day (0, 1, 3, 6). For all of Treatment 5 samples, there is a decreased level of RSI compared with Treatment 1. Furthermore, Treatment 5 looks similar to the controls.)

6.0 Discussion

Physostomous juvenile Chinook salmon, an ESA-listed species, have been shown to experience a range of injuries from very mild, such as hematoma in fins, to very severe, such as mortality from formation of gas bubbles in the gills leading to suffocation. These injuries have been observed following exposure to rapid decompression (Stephenson et al., 2010), pile driving impulsive sound (Halvorsen et al., 2011 In press), and intermediate-duration sounds generated by confined underwater explosions (Carlson et al., 2011). This project investigated the consequences on juvenile Chinook salmon from long-duration exposure to simulated tidal turbine sound. The 24 hour noise exposure should be considered a worst case-scenario for this species because they are migrant fish and it would be assumed that they would continue on their route and 'pass' by turbines. Also the sound levels generated by tidal turbines are influenced by the rotation speed and therefore the generated sound level would have more variability and thus a higher probability of being lower than the levels presented in this study. Alternatively, for a resident species, such as a bass that reefs onto man-made structures, 24 hours would not be a worst case scenario.

Electrophysiological testing of the auditory system indicated no effects on hearing sensitivity. This suggests that as Chinook salmon pass very near to a turbine they would not experience changes in their hearing sensitivity. Furthermore, as the fish are farther away from a turbine there is even less risk to the auditory system as the sound level would decrease on an order of 6 dB per doubling of distance from the turbine.

The preliminary analysis of the tissue damage data indicated a low level of tissue damage. The salmon used for this study were slowly pushed into an early smolting process (which is not often done), over a 3-week time frame and allowed an additional 2 weeks to adjust to smoltification before experiments began. It appeared that the initial treatment exposures (T1 and T2) had the highest RSI levels (amount of tissue damage) (Figure 5.1) and the RSI slowly decreased over time (and treatments) for each treatment, which suggests that variables other than sound are also factors. In any case, the injury responses from the juvenile Chinook were minor injuries that had a low physiological cost to the fish. Likewise for hearing sensitivity, 24 hours would be an extreme exposure for a migrant species like Chinook and as the fish are farther away from a turbine there is even less risk of damage to tissues.

Overall, the sound level from this study would be an extreme exposure situation for migrating Chinook salmon and the results indicated low levels of tissue injury and no effects on hearing. Collectively this means that Chinook salmon may be at a relatively low risk of injury from tidal turbines located in or near their migration path.

7.0 Ongoing Studies of Acoustic Effects on Fish

The project associated with the Snohomish PUD turbines will be completed in late 2011. However, under other Department of Energy Water Power Program funding, PNNL will continue to investigate effects of underwater noise on native fish in Puget Sound. These findings will provide additional insight and information on potential effects on fish from tidal turbines. The major activities under the DOE project in FY 2012 are to complete analysis of the juvenile Chinook salmon data acquired in FY 2011, test the response of a marine physoclistous species to an exposure from tidal turbine sounds, and use the fish index of trauma (FIT) model results and behavioral models for juvenile Chinook salmon and a physoclistous species to perform analyses that characterize the risk of exposure to tidal turbine sounds for these species.

Consideration has been given to the selection of a physoclistous species for evaluation of physiological response to exposure from tidal turbine sound. The leading candidate at this time is a rockfish. *Sebastes* represents a number of rockfish species in the Sebastidae family that are resident in Puget Sound. Species in this family are of concern because they are ESA-listed in the Puget Sound area. Sebastes species are marine; behaviorally they prefer to 'reef,' meaning they would be likely to aggregate and live on and in close vicinity of the tidal turbine structures. In addition, their physiology is different from that of the salmonids. *Sebastes* spp. are physoclistous; they use a dense capillary network to move gas from the blood to fill their swim bladder. When these fish are in an area of intense pressure waves, their swim bladder is closed and they are not able to volitionally "release" gas from their swim bladder as can salmon to deflate the swim bladder and thereby avoid some types of barotrauma. Depending on availability of *Sebastes* spp., testing could begin during quarter 2 of FY 2012. The experimental design will parallel the study on the juvenile Chinook salmon.

If *Sebastes* spp. or any other similar reef species are not available, staff will consider the use of a surrogate or a candidate elasmobranch species (sharks, skates). Elasmobranches, specifically skates and rays, often are found buried in the substrate, and these animals are without a swim bladder so they are also physiologically different from salmonids. Because turbines are coupled to the substrate, the noise generated from the turbines will travel along the sea floor for some distance. These substrate vibrations will be detectable by the elasmobranchs, but it is uncertain if they would be negatively impacted by exposure to substrate-borne sound. Because sharks and skates are generally not available from commercial suppliers, it may be necessary to procure wild-caught organisms to support laboratory testing.

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