APPENDIX A

BAROTRAUMA INJURIES

Table A-1 lists all potential barotrauma injuries that could have resulted from exposure to pile driving sounds.

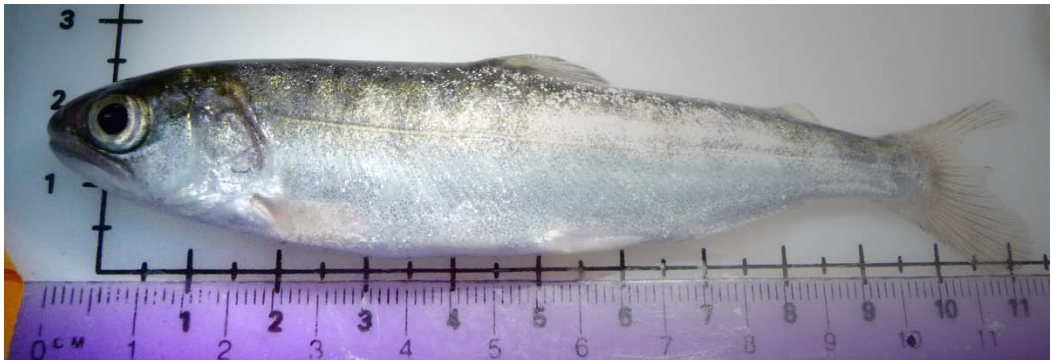
Table A-1. Potential barotauma injuries

External	Internal
Anal fin embolism	Abdominal blood source identification
Anal fin hematoma	Abdominal cavity filled with blood
Anal fin hemorrhage	Bacterial kidney disease (BKD) - white spots
Blood or bile from vent	Body muscles hematoma
Blood spots on vent	Brain case, blood around brain
Caudal fin embolism	Bruised spleen hematoma
Caudal fin hematoma	Burst capillaries along kidneys and wall
Caudal fin hemorrhage	Damage to gall bladder
Dead within 1 hr	Fat hematoma
Dorsal fin embolism	Fat present or absent
Dorsal fin hematoma	Fully deflated swim bladder (no ruptures)
Dorsal fin hemorrhage	Heart beating upon opening
Exophthalmia (eye pop)	Hepatic (liver) hematoma
Ext. Sign of pericardial hemorrhage	Hepatic (liver) hemorrhage
Gill mild embolism	Intestinal hematoma
Gill severe embolism	Intestinal hemorrhage
Left eye embolism	Otoliths: aragonite (a) or vaterite (v)
Left eye hemorrhage	Ovaries/testes hematoma
Pectoral fin embolism	Partially deflated swim bladder (no ruptures
Pectoral fin hematoma	Pericardial (heart) embolism
Pectoral fin hemorrhage	Pericardial (heart) hemorrhage
Pelvic fin embolism	Pericardial hematoma
Pelvic fin hematoma	Raspberry looking spleen
Pelvic fin hemorrhage	Renal (kidney) anterior embolism
Right eye embolism	Renal (kidney) hemorrhage
Right eye hemorrhage	Renal hematoma
Stomach inversion	Renal mid embolism
	Renal posterior embolism
	Ruptured swim bladder anterior
	Ruptured swim bladder mid
	Ruptured swim bladder posterior
	Swim bladder hematoma
	Spleen hemorrhage

APPENDIX B

PHOTOGRAPHS OF INJURIES (NOT ALL-INCLUSIVE)

B.1 EXTERNAL INJURIES



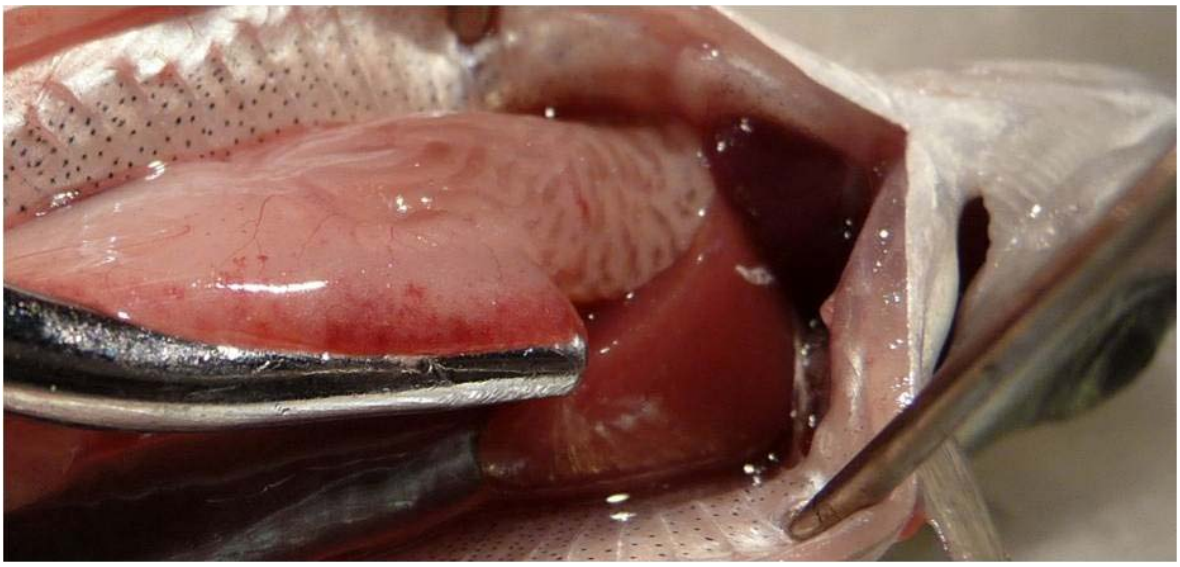
Control fish showing no external injuries

Blood spots on vent, pelvic fin hematoma

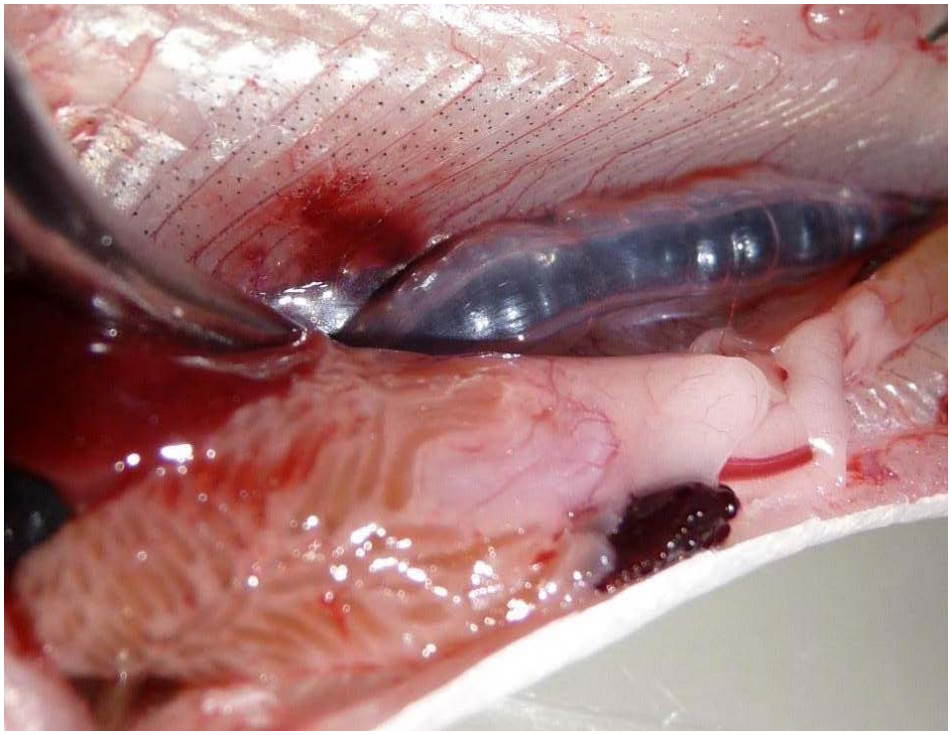
Eye hemorrhage

B.2 INTERNAL INJURIES

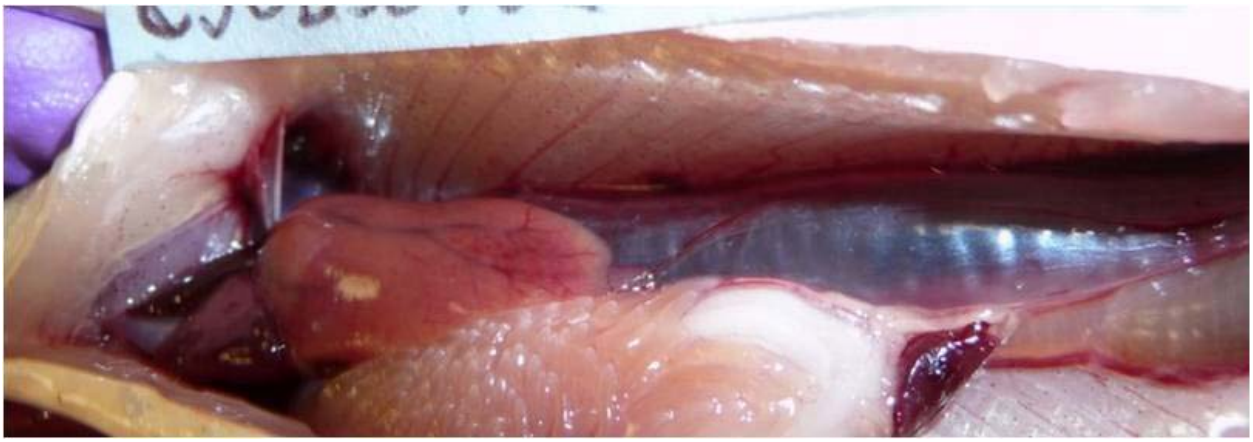
Control, healthy internal organs and tissues



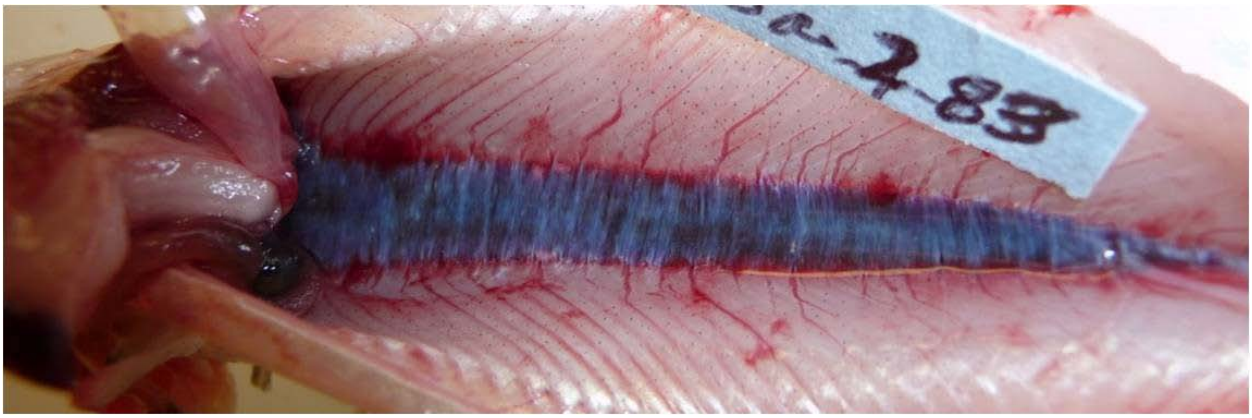
Fat hematoma



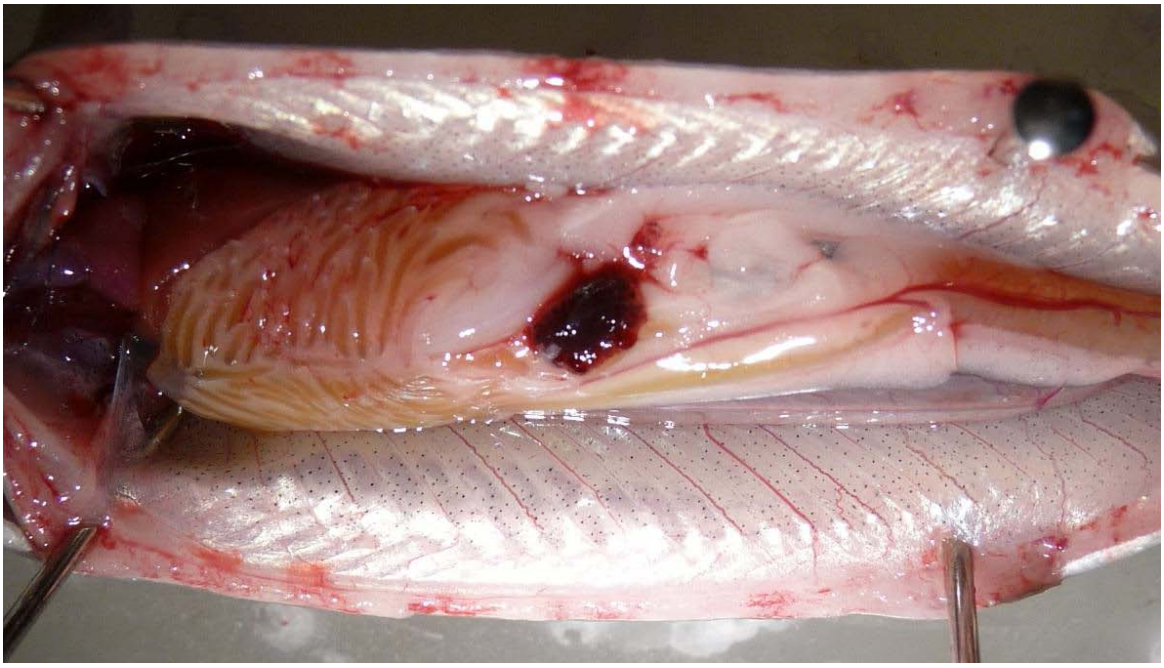
Body muscle hematoma



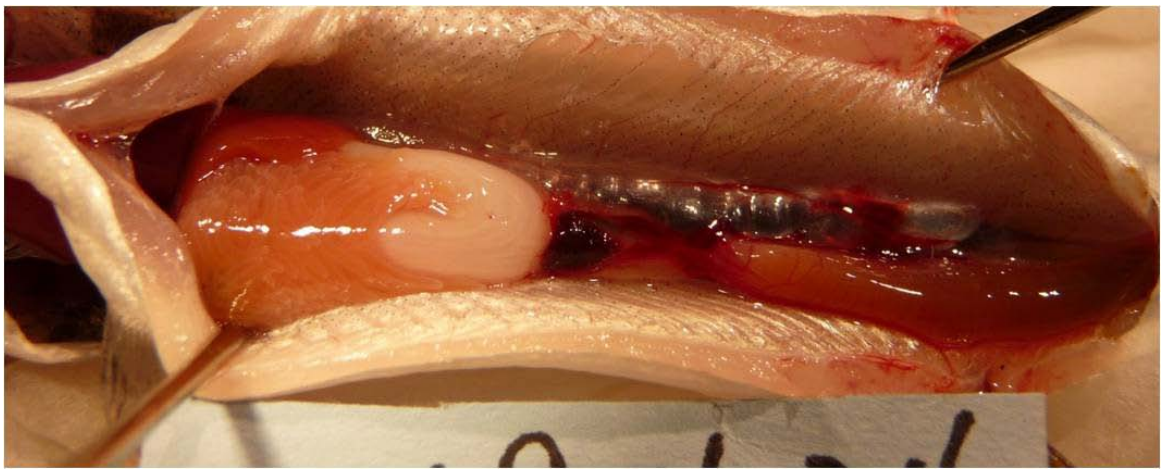
Cardiac hematoma, liver hemorrhage, deflated swim bladder



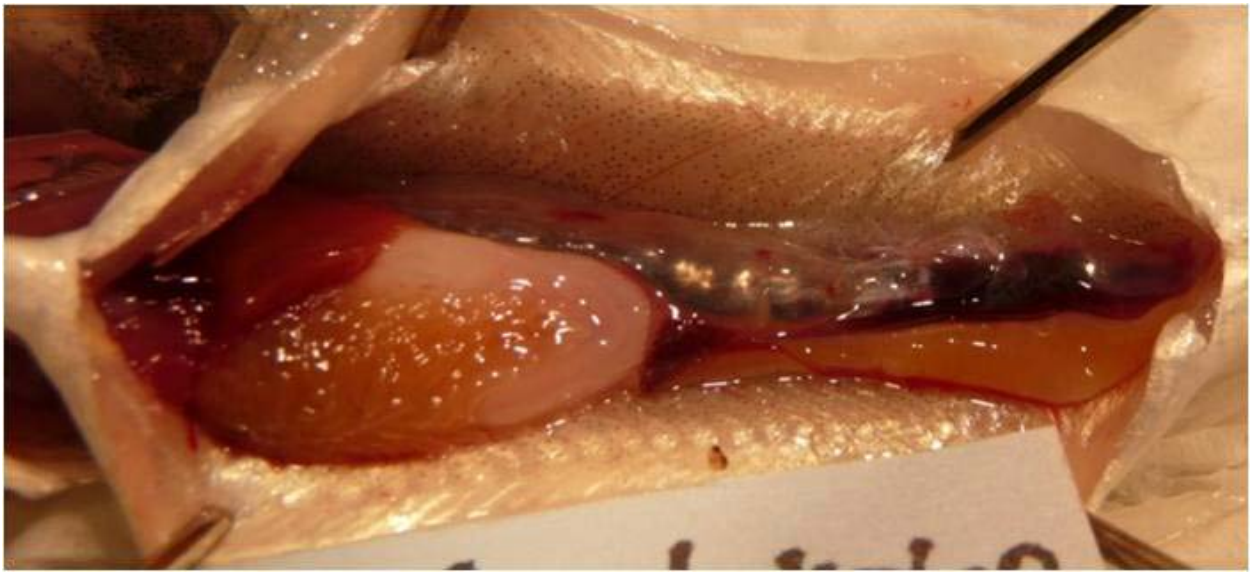
Kidney hemorrhage, burst capillaries along muscle wall



Spleen hemorrhage, intestinal hemorrhage

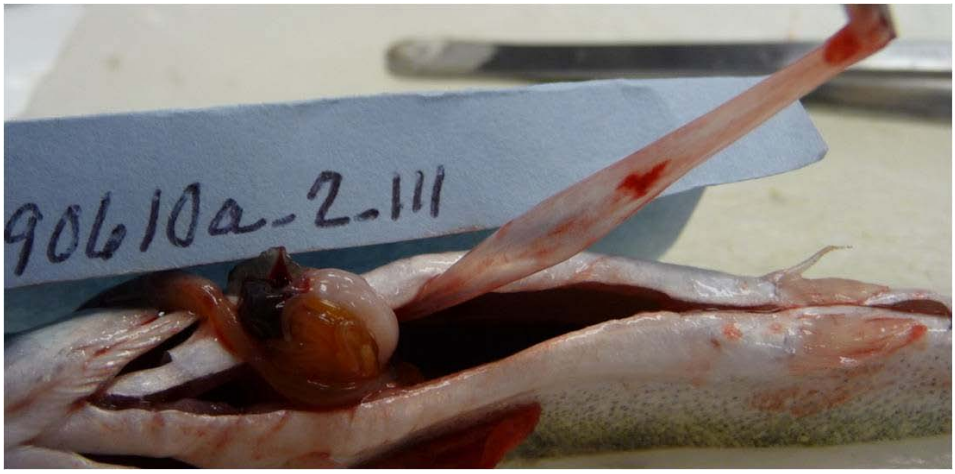


Ruptured swim bladder, intestinal hemorrhage



Ruptured swim bladder, intestinal hemorrhage

Partially deflated swim bladder without rupture

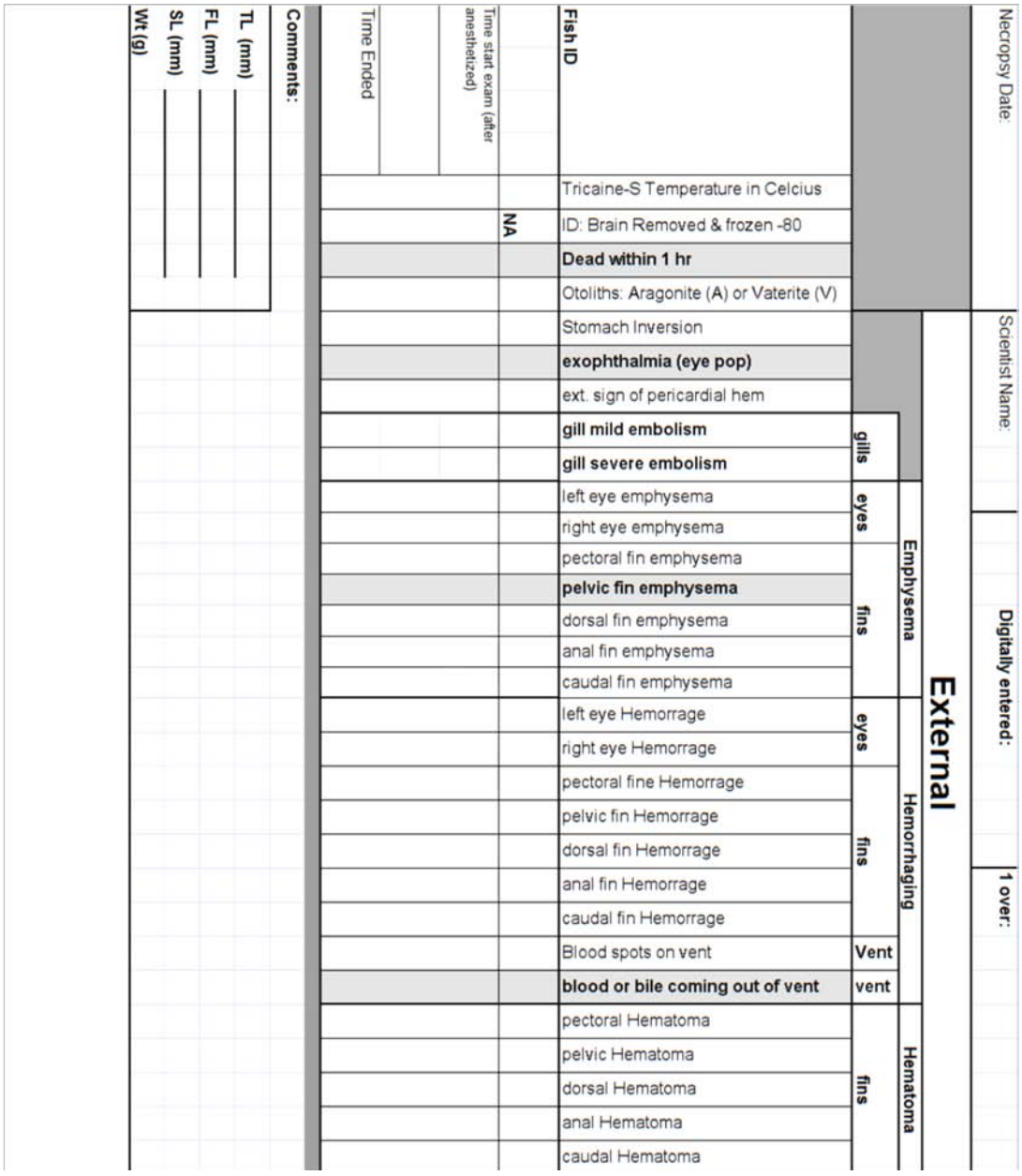


Swim bladder hematoma

Swim bladder hematoma

APPENDIX C

DATA SHEETS



Necropsy Date:

Scientist Name:

Digitally entered:

1 over:

External

Fish ID			
Tricaine-S Temperature in Celcius			
ID: Brain Removed & frozen -80	NA		
Dead within 1 hr			
Otoliths: Aragonite (A) or Vaterite (V)			
Stomach Inversion			
exophthalmia (eye pop)			
ext. sign of pericardial hem			
gill mild embolism		gills	
gill severe embolism		gills	
left eye emphysema		eyes	Emphysema
right eye emphysema		eyes	Emphysema
pectoral fin emphysema		fins	Emphysema
pelvic fin emphysema		fins	Emphysema
dorsal fin emphysema		fins	Emphysema
anal fin emphysema		fins	Emphysema
caudal fin emphysema		fins	Emphysema
left eye Hemorrage		eyes	Hemorrhaging
right eye Hemorrage		eyes	Hemorrhaging
pectoral fine Hemorrage		fins	Hemorrhaging
pelvic fin Hemorrage		fins	Hemorrhaging
dorsal fin Hemorrage		fins	Hemorrhaging
anal fin Hemorrage		fins	Hemorrhaging
caudal fin Hemorrage		fins	Hemorrhaging
Blood spots on vent		Vent	Hemorrhaging
blood or bile coming out of vent		vent	Hemorrhaging
pectoral Hematoma		fins	Hematoma
pelvic Hematoma		fins	Hematoma
dorsal Hematoma		fins	Hematoma
anal Hematoma		fins	Hematoma
caudal Hematoma		fins	Hematoma

Time start exam (after anesthetized)

Time Ended

Comments:

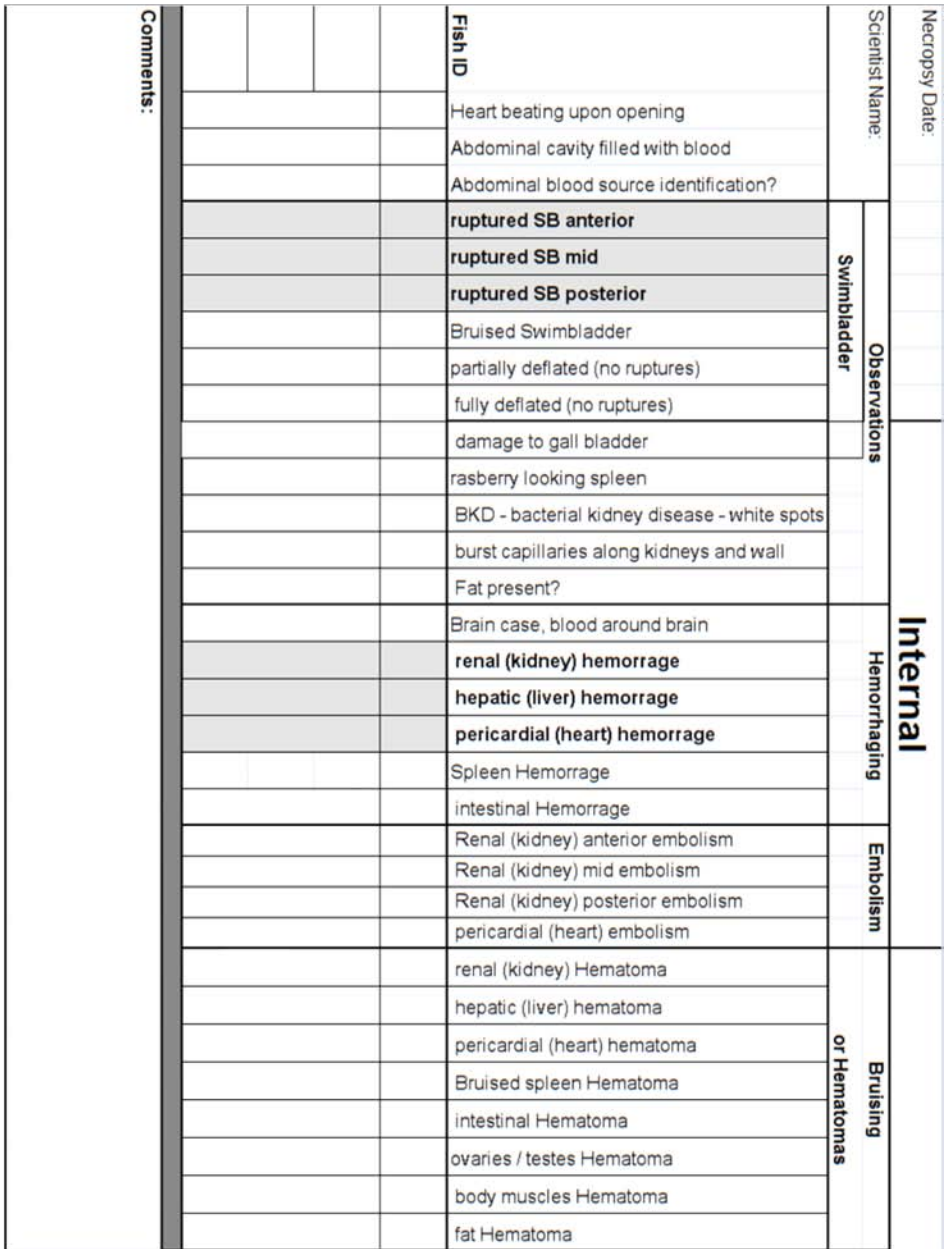
TL (mm) _____

FL (mm) _____

SL (mm) _____

Wt (g) _____

Barotrauma injury scoring sheet: external



Barotrauma injury scoring sheet: internal

Date (yyyy_mm_dd)				**Initials**	
	Calibration				
Start time			Freq (Hz)	Level tested (dB re 1 uPa)	
Tube temp			48	120	
J-11		psi	80	120	
B&K gain			125	120	
End time			250	120	
End temp			500	120	
			tab 10	background noise - input to J-11 disconnected	
	AEP				
		CSXX_yymmddx_#_xx			
File name:					
Ice on flange:					
	Order	**Freq**	**Threshold**	Initials	
		48 Hz			
		80 Hz			
		125 Hz			
		250 Hz			
		500 Hz			
monitor					
Bucket Temp/time:			T/T:		T/T:
HICI-ft end time					
Start time			**Flaxedil dose:**		
Start temp				**TL (mm)**	
J-11		psi		**Fork length (mm)**	
End time				**SL (mm)**	
end temp				**WT (g)**	
J-11		psi			
			Otoliths	AA AV VV	
Home tank temp				A= aragonite (normal)	
				V=vaterite (abnormal)	
Notes:					
					initials
Fish gilling after removed from AEP testing?				**Digitally entered**	
	Yes No			**1 over**	

AEP data sheets

fill tube w/ 12.5 degree water & T. Bucket		stop video	DATE:
all bubbles out		turn off power amplifier	
lower shaker lid		turn off blowers	Initials:
clear bubbles		Deflate Feet	
cooling blower on			
blowers warm up for 15 min		If run another fish:	
wear ear protection whenever blowers are on			
fasten lid		HICI-FT into verticle	
inflate vibration isolation feet		open lid- install safety strap & put on tension	
		take temp	
randompile		remove fish	
randomcomp_yyyy_mm_dd_hhmm.mat		bring fish to AEP person	
longexposureVI		save video	
SRS560 matches gain setting		note video MZL file name	
EightSignalSEL.dat			
Crown Amp On		drain water	
calculate SELcumm		refill with conditioned water @ 12.5 degrees	
set PA4s		remove all air bubbles	
shut off blowers/amp		lower lid into water	
ready video, marantz		remove air bubbles off lid	
ready randompile.dat		Inflate vibration isolation feet	
		place mesh over tube opening	
Break seal		place fish into acrylic tank	
open shaker lid			
clear bubbles - if a lot - redo SELcumm measure.		If done for the day:	
mesh over tube opening			
temps		stop video	
		turn off power amplifier	
Ice in bin for fish bucket		turn off blowers	
signs on doors!			
fish acclimation		deflate feet	
temps (Max +/-1 deg; to 14 allowed)		HICI-FT into verticle	
		open lid - install safety strap	
lower shaker lid		take temp	
clear bubbles		remove fish	
fasten lid		bring fish to AEP person	
empty acrylic tank		save all videos, & audios	
empty drain hose thoroughly			
all wheels clear the floor		unplug camera	
HICI-FT into horizontal		turn off laryngoscope/light source	
level HICI-FT		turn off SRS560, & marantz	
		set PA4s to 30	
Cooling blower running and hooked up		drain water	
turn on blowers		dry out tube inside and out	
turn on power amp		dry off top of shaker	
start video, marantz		shut down all software programs	
start exposure		refill reservoir	
do not refill reservoire		dump thermometer water	
bucket temp to 13.5 to 14.5 (use ice)			

HICI-FT: Checklist for each day

Date (yyyy_mm_dd)		Initials	
Time started filling tube with water			
Time blowers on (need 15 min warm up)		Time blowers off	
air out of tube	yes no		
Time			
Temperature			
TGP			
Matlab			
randompile	yes no		
randomcomp_yyyy_mm_dd_hhmm.mat			
Labview			
longexposureVI	yes no		
EightSignalSEL.dat	yes no		

DAQ		**File name**	
points per channel		**PA4**	
desired scan rate		**SEL**	
Hydrophone		Peak + SPL	
sensitivity	-212	Peak + Pa	
gain - match SRS560		Peak - SPL	
Waveform Generation		Peak - Pa	
strike number		desired # pile strikes	1920
seconds		240 log*10 + SEL = SELcumm	
desired update rate		**SELcumm =**	
Digital gain			

DAQ		**File name**	
points per channel		**PA4**	
desired scan rate		**SEL**	
Hydrophone		Peak + SPL	
sensitivity	-212	Peak + Pa	
gain - match SRS560		Peak - SPL	
Waveform Generation		Peak - Pa	
strike number		desired # pile strikes	1920
seconds		240 log*10 + SEL = SELcumm	
desired update rate		**SELcumm =**	
Digital gain			

DAQ		**File name**	
points per channel		**PA4**	
desired scan rate		**SEL**	
Hydrophone		Peak + SPL	
sensitivity	-212	Peak + Pa	
gain - match SRS560		Peak - SPL	
Waveform Generation		Peak - Pa	
strike number		desired # pile strikes	1920
seconds		240 log*10 + SEL = SELcumm	
desired update rate		**SELcumm =**	
Digital gain			

HICI-FT: Data sheet to set level each day

Date (yyyy_mm_dd)				Initials	
mesh over tube opening	yes no				
pre fish:	**Time**				
	Temperature				
	TGP				

PUT SIGN ON BOTH DOORS BEFORE RELEASING FISH

Time fish in acrylic tank		use continuous sampling observation method			
20 min acclimation time	**SalmonCD**	Fish ID			
	Event #	**Time**	**Describe w/ buoyancy**		
	1				
	2				
	3				
	4				
	5				
	6				
			final buoyancy =		
Was fish corraled into tube?	y n				
	SalmonCV	Fish ID			
	event #	**time**	**describe**		
	Event #	**Time**	**Describe w/ buoyancy**		
	1				
	2				
	3				
	4				
	5				
	6				
			final buoyancy =		
Was fish corraled into tube?	y n				
Time fish entered into tube					
	Time				
	Temperature				
	TGP				

Notes:

HICI-FT: Data sheet for fish acclimation

Date (yyyy_mm_dd)				Initials	
Labview					
	longexposureVI	yes	no	Salmon CD Fish ID	
	randompile.dat	yes	no	Salmon CV Fish ID	
DAQ				**File name**	
	points per channel			PA4	
	desired scan rate			**SEL (96) - measured**	
				SEL (1920)cumm - calculated	
Hydrophone				Peak + SPL	
	sensitivity	-212		Peak + Pa	
	gain - match SRS560			Peak - SPL	
				Peak - Pa	
Waveform Generation				desired # pile strikes	
	strike number			20 log*10 + SEL = SELcumm	
	seconds				
	desired update rate				
	digital gain				
Exposure	**start time**			**signal end time**	
Final	**Time**			**Bucket temperature**	
	Temperature				
	TGP				
Video	temp file name				
	final file name				

HICI-FT: Data sheet for pile driving exposure information

Date (yyyy_mm_dd)		Initials	
	MARANTZ		
SRS 560			
	Gain		
	filter: high pass 6 dB	3 Hz	
	Filter: low pass 6 dB	1 kHz	
	coupling	AC	
	gain mode	low noise	
	channel	A	
	signal	inv	
Hydrophone			
	sensitivity	-212	
Marantz Settings			
	(DO NOT CHANGE settings)		
	Record level setting	3	
		PCM	
	file type	.WAV	
	sample rate	16 kHz	
	input	line L mono	
	hydrophone into Lt RCA input		
	number of fish in tube		
	time duration of exposure		
	file number on card:		
	new name for sound file:		
	PA4		
	SEL (96) - measured		
	SEL (1920)cumm - calculated		
	# pile strikes		

HICI-FT: Data sheet for Marantz sound file information

APPENDIX D

PROTOCOLS AND OTHER DOCUMENTS

Tail-Clipping Protocol

Popper Lab: Aquatic Bioacoustics

April 2009

Materials

- Buffered MS-222
- Bucket dedicated for use with MS-222
- Hole-punch
- Fish nets
- Stress Coat or PolyAqua water conditioners
- Small liquid container of stress coat/PolyAqua to dip hands and nets into
- A second bucket for fish recovery which has Stress Coat in it
- Latex gloves

Procedures

The purpose of doing these tail clippings is to be able to distinguish two fish from each other while they undergo the exposure portion of our experiment, as well as the procedures following. The clipping will be done on Fridays when we prepare the following week's test subjects by moving them from the home tank (tank 5 @ 12°C) to the holding tank (tank 4 @ 14°C). Since we will be moving *all* fish from tank 5 into tank 4; all fish need to be handled exactly the same. Including fish that will not be tail clipped.

1. PUT ON LATEX GLOVES
2. Make your anesthetizing solution
   a. Mix buffered, concentrated MS-222 solution using the following recipe:
      i. 20g MS-222
      ii. 1g $NaCO_3$
      iii. 500ml H_20
   b. Add 10 ml of concentrated MS-222 to 10 Liters of water from tank 5 into the bucket dedicated for use with MS-222 only; resulting in 20 mg/l concentration.
   c. Stir
3. Prepare the "recovery" bucket
   a. Add Stress Coat or PolyAqua to a second bucket.
   b. Follow manufacture's dilution rates, but ensure that there is about 2 gallons of water in the bucket.
   c. Use water from the tank that the fish will be going into (tank 4).
4. Prepare the dipping solution
   a. Use a smaller liquid-safe container and mix a concentrated solution of Stress Coat or PolyAqua at a ratio of about 1:5

b. This will be used for coating the net, hands, hole punch, and fish during the actual clipping, so you will only need about ¼ a liter.

5. Anesthetize the fish

a. Capture all fish that need to be handled/moved, including the fish to be clipped and those that will not, and place gently into the MS-222 bucket.

b. Watch very closely
   i. You do not want to over anesthetize the fish by exposing them to MS-222 for too long nor clip fish that are not yet anesthetized.
   ii. As soon as the first fish is "drowsy" or "lazy", and flipped on its back, begin clipping.
   iii. You will need to watch each fish individually—if one is particularly effected by the MS-222, clip that one next and get it out of the solution.
   iv. Do not clip fish until they are anesthetized.

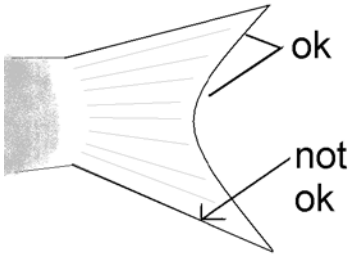
6. Clip the fish

a. Capture the drowsiest fish from the MS-222 bucket using the net

b. Holding the fish and net over the container, drizzle dipping solution over the fish, hands, hole punch, and net

c. Hold the fish in your hand with its head away from your thumb so that the tail is exposed. It may be easier to hold the fish using the net—as in grab from outside the net while the fish is in the net.

d. Use the hold punch to clip appropriate part of the caudal fin (caudal dorsal or caudal ventral). Do not clip outermost part of fin—stay within the dorsal and ventral edges of the caudal fin.



7. Release into recovery bucket

8. Repeat for all fish to be clipped

9. For fish that will not be clipped, do **every** step except actually squeezing the hole punch. This includes holding, drizzling, and even pinching the caudal fin with your fingers to simulate the hole punch.

10. Watch fish for recovery

a. Once all fish are back to normal levels of activity, which should take about 5 to 10 minutes, release them into tank 4 **using the net**. Do not dump the recovery bucket water into tank 4.

11. Record on fishcare sheets the total number of fish moved and in parenthesis the number of each clipping (CD, CV, NC)

IACUC Protocol Approval

UNIVERSITY OF MARYLAND

GRADUATE STUDIES AND RESEARCH
Institutional Animal Care & Use Committee

W. Ray Stricklin
IACUC Chair
wrstrick@umd.edu
Phone: (301)405-7044

May 20, 2010

Dr. Arthur N. Popper
Department: Biology
University of Maryland
apopper@umd.edu

Dr. Popper,

This letter is to inform you that on **May 20, 2010**, the members of the Institutional Animal Care & Use Committee (IACUC) reviewed and approved the annual review for the protocol:

Effects of pile driving sounds on fish

R-09-23

Please note that an approved protocol is valid for three (3) years unless there is a change in the protocol. Thus, this protocol is valid until **June 15, 2012**. Federal laws indicate that protocols must be reviewed yearly. You **must** submit an annual review in **May 2011**. All work extending beyond the approval date of the protocol must be submitted to the IACUC as a new protocol.

Sincerely,

W. Ray Stricklin
Asst. Dean, College of Ag. & Natural Resources
Chair, IACUC

CC: Doug Powell, Amanda Underwood

HICI-FT Protocol

Fish Capture

Primary goal is to limit startling fish as much as possible, this is very important to keep stress levels down and maybe some air in their swimbladders.

In Fish Room

1. Turn off or remove bubblers in tank
2. Have a bucket ready with some water from the fish tank inside
3. USE TWO nets
4. Move SLOWLY, so as not to scare fish
5. Position nets (one in each hand) wide apart – to edges of tank
6. Slowly lower nets into water until reach the bottom
7. Slowly heard a couple fish toward you and into nets
8. If fish swim into a net – bring nets together to close off opening
9. Slowly bring fish up to surface
10. Gently place the nets/fish into the bucket.

In HICI-FT Room

11. Carry upstairs to 3228
12. Place bucket onto foam in 3228
    a. if ‘not ready’ for fish, then put aeration into bucket immediately.
13. When ready:
14. Slowly capture each fish out of the bucket using a net
15. Release fish gently into water of the plexiglass area of HICI-FT

HICI-FT Protocol

Start the day by:

1. Fill the HICI-FT tube with conditioned water at 12.5°
    a. At end of experimental day only refill the reservoir tank
    b. Fill thermostat bucket
2. Warm shakers by turning on blowers/field coils for 15 min (minimum)
3. Turn on cooling blower and force air into top shaker
4. Blow away ALL air bubbles in tube using turkey baster while filling
5. make sure to jet water into all through-hull fittings these are areas that hold air bubbles well
    a. camera
    b. hydrophone
    c. bolts
    d. seams
    e. bolts, everywhere
    f. large o-ring
6. When water level reached the mark on acrylic tank then lower the top shaker (lid)
7. Don't put arms under lid once lowered, not safe!
8. Blow air bubbles off of lid surface (bottom) using turkey baster,
9. Make sure to jet water into all seams and bolts.
10. After all air bubbles are removed:
11. Slowly lower lid until the pins are lined up into the holes
12. At this point look at the face plates of the shaker lid again – any more bubbles?
    a. If so, blow them away!!! (NO AIR in TUBE!!!)
13. Gently lower lid all the way down
14. Hand tighten shoulder bolts (using tool) over metal lip; snug not too tight
15. Fill vibration isolation feet to 50 psi – NO MORE! (there is red arrow marking max psi on the hand pump)
16. Mark the temperature, TGP, and time in the book

To begin, we need to measure SEL of the signal:

Matlab

In Matlab following >> type in commands as written here:

17. Open Matlab program

In Matlab following >> type in commands as written here:

18. Directory: C:\hici-ft_files\jims VI\ajimsmfi\fishstuff
19. >>randompile
    a. This saves the pile driving signals in random order – called randompile.dat
        i. This is given the same name daily so follow to next step.
    b. A graph of the 96 pile signals is shown & calcs on the workspace are shown too.
20. >>Save randomcomp_yyyymmdd_hhmm.mat
    a. Use todays date: yyyymmdd
    b. Time (hhmm): uses 24 hour time clock: military time
    c. This saves all the information contained within the randompile.dat file (p1, p2, … porder)
    d. randomcomp_yyyymmdd_time.mat
    e. file is save into a different directory
        i. c:\hici-ft_files\eagleharbor
21. Exit out of matlab

To measure approximate SEL in tube

Labview

In labview open

22. Open Labview 8.5
23. Click on link to file: c:\... transdrive_longexposure.vi
    a. This shows the front panel for Longexposure VI
    b. The panel will have a blue background
    c. IF panel color is red, the wrong program has been opened
        i. Close VI and open the proper one.

Device Setup Tab

24. Value Areas with a green background: Change these values when needed
25. Value Areas with a white background: Do NOT change these values
26. At the top right is a file folder symbol (where the files are selected)
    a. Click on folder
    b. Select file: EightSignalSEL.dat
    c. Click OK
27. Set the hydrophone setting on SRS560 amplifier to needed gain level (usually 2)
28. Enter the hydrophone gain on front panel (to match the SRS560 value)
29. Points per channel = 60000

30. Desired scan rate = 5000
31. Number of pulses = 1
    a. This will be one presentation of each of the 8 pile signals (one file)
32. Signal length = 12.000
33. Desired Update rate = 20000
34. Digital gain = 10
35. PA4s both set to the same value; start at 8.0
36. Make notes in lab book of all settings
    a. From step 23-35
37. (Blowers are already on)
38. Turn on Crown Power amplifier
39. To run program;
    a. Click the white arrow at the top left corner of the screen.
    b. Click on Measured signal tab

Measured Signal Tab

40. Write down the file name found at the top left
41. Write down the total SEL value
42. Write down the peak pos & peak neg SPL and corresponding Pa values
43. Make sure there isn't clipping in the Measured tab window (>5V)
44. Calculate the SEL_{cum} value for the number of pile strikes to be done for that day.
    a. $SEL + 10\log N = SEL_{cum}$

    EXAMPLE:

    b. SEL value for 8 piles signals was 191.923 (from labview)
    c. N=240 (x8 is the number of pile strikes in our file) = 1920
    d. Our desired SEL_{cum} is 220
    e. Into calculator: 240 log * 10 + 191.923 = 215.725
    f. SEL is too low need to turn down the PA4s
45. Rerun steps 34-45 until SEL_{cum} =220, then leave the PA4s set at last value using calculations to attain 220 SEL
    a. Write down file name and PA4 settings for each rerun
46. Turn off blowers/field coils
47. Leave cooling blower on

48. Slowly open lid
    a. Loosen shoulder bolts until they swing away
    b. gently rock the lid to 'break' seal
    c. lift lid slightly above the pins
    d. blow away air that is on lid
    e. Write Temperature and TGP from INSIDE THE TUBE –

Video

49. Ready softwares:
    a. Open the Movie Star software (yellow star icon)
    b. Click on capture
        i. Select device (DVC 150)
50. Cover tube opening with mesh
51. Turn on Marantz.
    a. Slide on/off button on top to turn on.
    b. Do not change any settings on the unit
52. Fill Vibration isolation feet
53. Get ice in bin from room (big grey door on 3rd floor room 3268) for cooling salmon water
54. Catch the salmon (see steps above)
55. Record time, temperature, TGP
56. Gently Place 2 salmon into plexiglass tank area
57. Allow 20 minutes for filling swimbladder and becoming neutrally buoyant
58. Observe fish for filling swimbladder (gulping air at surface) – continuous sample
    a. Write in lab book
        i. Each time fish gulp air
        ii. ID fish with the time
        iii. Write attitude of fish: is neutrally buoyant or not
59. After 20 minutes
60. Move SLOWLY and remove mesh and allow fish to go into tube voluntarily
    a. Generally they go on their own they like the dark
    b. DO NOT SCARE FISH,
    c. MOVE SLOWLY,
    d. DON'T MAKE EYE CONTACT OR LOOK AT FISH
    e. Above actions will make fish burp out air from swimbladder
    f. If fish won't go into tube on their own, very very slowly and gently herd them toward the tube opening using a net – near their tail.

61. Record time and temperature, TGP, of water inside the tube
62. When fish is inside tube, make sure there is no air on the shaker lid
63. Slowly and gently, lower the lid into place following steps 8-15; watch for fish location.
64. Drain water out of plexiglas area by pulling out the sink drain.
    a. Capture the water into buckets
    b. Throw water away, do not reuse
    c. Make sure clear drain hose is empty of all water! – or it will end up on top shaker during rotation (not good).
65. Capture loose water in plexiglas area using sponge
66. Release the winch -wire cable from the shaker
67. Attach the wire cable to the side of the HICI-FT frame so the cable doesn't unspool
    a. If cable loosens on spool it will need to be rewound very tightly and orderly!
68. Use the tall silver bar to turn the wave-tube to the left.
    a. Only use the silver bar to rotate the wave-tube
    b. Never grab the Plexiglas to make adjustments
    c. Make sure all tubes, wires, etc are clear as making rotation, and have no strain
69. Keep drying out the Plexiglas container.
70. Put the HICI-FT wave-tube completely in a horizontal plane.
71. Re hook up the cooling blower once tube is horizontal.
72. Wheels of carriage should just clear the floor (1/8" clearance) – adjust vibration isolation feet as needed.
73. Use the level to adjust tube.

Labview

Device Setup Tab

74. In the longexposure VI
75. Open file named randompile.dat
76. Match the hydrophone setting on front panel to the SR560 amplifier
77. Points per channel = 720,000
78. Desired Scan rate = 5000
79. Number of pulses = (enter appropriate number)
80. Signal length = 144.000000
81. Desired update Rate =20,000
82. Digital gain = 10

83. PA4s both set to the value determined in step 46.
84. Write all your setting in lab book!
85. Turn on Equipment
    a. Turn on both blowers/field coils
    b. Turn on crown power amp
    c. Cooling blower turned on and in place over the top shaker
    d. Put in your hearing protection while equipment is on.
86. Do a check
    a. Hydrophone gain on front panel matches SR560?
    b. Are both PA4s set on predetermined value (check the value)?
    c. Both blowers are on and working –can you feel the air going thru hoses?
    d. Is the power amplifier on?
    e. Are the number of pulses set properly?
    f. Is the signal length set properly?
    g. Is the points per channel set properly?
    h. Has the randompile.dat file been loaded?
87. 86a-h: go thru CHECK list 3 TIMES!!!
88. Ready?
89. Turn on the laproscopic light source – about ½ brightness
90. Turn on Blowers
91. Turn on Power Amplifier
92. Start the video (click red button)
93. Start Marantz – push record button (to capture hydrophone signal)
    a. Record track #
94. Start labview randompile : Click the white arrow at the top left to run the longexposure VI
    a. This will present x number of pile signals
        i. To abort program click the red button at the top left
        ii. In emergency can also turn the crown power amplifier off.
    b. If control fish
        i. All equipment needs to be on
            1. Blowers/field coils
            2. Not the Power amplifier (STAYS OFF)
            3. Open the programs
            4. Run the randompile.dat file

Measurement Tab

95. Write the file name in the lab book found at top right.
96. Stay in room during experiments
97. Keep both doors closed during experiments – so sound doesn't affect our neighbors.
98. Write all information found on this page into lab book
    a. $SEL_{measured}$
    b. SELcaluculated
    c. Peak + SPL and Pa values
    d. Peak – SPL and Pa values
99. Give each fish an ID
    a. CSCD_yyyy_mm_dda
        i. CSCD =Chinook Salmon Caudal Dorsal tail clip
        ii. CSCV= Chinook Salmon Caudal Ventral tail clip
        iii. (a,b,c to denote 1st, 2nd, 3rd, etc trial for the day)
        iv.
100. Keep an eye on bucket water temperature
    a. Take temperature of bucket water
    b. Is it 14 degrees?
    c. If not, keep on ice 14 degrees (allow 14 +/- 1degree)
    d. Make note of final bucket temp
101. When completed mark the time in 24 hr scale
102. Turn off equipment
    a. Stop video by clicking square black button
    b. Blowers
    c. Power amplifier
    d. Cooling blower
    e. Marantz- hit the stop button on top right
    f. Video light source
    g. Deflate vibration isolation feet
    h. Unplug the video camera
103. Using the long silver handle, slowly turn the tube into a vertical position
    a. Make sure hoses and cables are clear, continuously
104. Loosen the shoulder bolts until they slide off the lid
105. No need to rock the lid to break the seal, without water surrounding the seal breaks easily.

106. Grab the winch-cable
    a. Make sure the cable is wound properly and tightly on spool
    b. Must be tight
    c. Must be in order, no crossing of cables on spool
107. Hook winch-cable onto top shaker, keeping tension on cable so it doesn't come loose on spool!
108. Raise the top shaker
109. Put the blue safety strap in place and back off a bit on the winch tension
    a. Share tension between cable and blue safety strap
110. Write down time and temperature and TGP inside the tube
111. Gently catch fish with net and place into bucket.
112. Bring fish to AEP person – use center stair case.
113. DO NOT tell person what treatment was done on fish – they need to be 'blind'.
114. Give them fish ID
115. Save video, go to the Movie Star software
    a. Click edit tab @ top left
    b. Click titles tab at right
    c. Create tab- to create a new title (include all info below)
        i. Name of fish (Salmon XX#)
        ii. SELcumm_221.234
        iii. Date (YYYY_MM_DD)
        iv. XXXX_pile strikes
    d. Click save button on bottom right (saves title page you made)
    e. Drag new title page into 1st frame of film strip at the bottom
        i. Program automatically shifts to movies
    f. Drag the days movie.mzl into the 2nd frame of film strip
    g. Click Produce @ top left
        i. All settings are for .mpg
        ii. Click Render
            1. This will make the movie into .mpg
            2. Process /save time will equal duration of recording
                a. If movie is 1 hour, rendering will take 1 hour
116. Save video on all experiments

At the end of the day

117. Turn PA4s to 30.0
118. Turn off SRS560
119. Turn off Marantz

120. Turn off laryngoscope light source
121. Unplug camera
122. Make sure cooling blower is off
123. Close the video program (after its done saving movie)
124. Close the labview program
125. Turn off computer
126. Make sure vibration isolation feet have been deflated
127. Make sure water reservoir has been refilled
128. Thoroughly dry out inside HICI-FT tube
129. Dry off top shakers bottom surface
130. Dry out acrylic tank
131. Dump thermometer bucket water
132. Make sure all buckets are empty
133. Straighten up, etc.

Trouble shooting notes

Shakers need to be turned on for 15 minutes before running signal – they need to warm up to generate consistent signals.

Barotrauma Protocol

Tools needed

- Other tools for general barotrauma necropsy (dissecting scope, scissors, etc.)

To euthanize

- After treatment euthanize fish in buffered MS-222: 80-100 mg/L concentration
- Leave fish in euthanizing bath until opercular movement ceases, less than 10 minutes.
- Remove fish from bath.
- Place fish on dissection table
- Proceed with barotrauma necropsy.

Barotrauma Necropsy Protocol

Purpose:

This part of the project is to determine the extent of barotrauma on salmon after exposure to pile driving signals in the HICI-FT. Salmon acclimated to the HICI-FT holding area, were exposed to pile

driving signals in the HICI-FT, moved to the HAT to evaluate their auditory system, then underwent necropsy to determine the degree of barotrauma injury incurred.

Before the necropsy begins, the AEP person supplies Fish ID on a piece of colored tape along with the fish to the person conducting the necropsy.

Detailed Description of Necropsy:

Necropsy is divided to two general areas; External and Internal signs of injuries on tissues and organs.

External exam:

1. **Eyes** – look for any signs of blood or presence of bubbles (embolism). Open the mouth and examine the underside of the eye through the roof of the mouth, roll the eyes to look for any signs of bubbles or blood through the tissue of the mouth.
2. **Stomach protrusion** – Open the mouth of the fish and look down its throat to see if the stomach is being force into its oral cavity.
3. **Gills –** Use forceps, open the operculum and examine each gill raker. Look for bubbles along the filaments from the base out to the tips of the gills. It is advised to examine the gills under a dissecting scope.
4. **Hemorrhage in pericardial cavity** – Under the final gill raker, closest to the body of the fish, is the pericardial window. The tissue surrounding the cavity is thin enough to see through. Examine for bubbles or pooled blood in the internal pericardial cavity.
5. **All Fins** – Examine each fin by pulling the leading fin ray to stretch them out. It is best to gently wipe the fin surface to eliminate any superficial bubbles. Look for bubbles inside the fin rays that can't be wiped away, and look at the base of the fin for bubbles or blood pooling. All fins should be examined closely for bubbles and/or blood.
6. **Anal pore** – Before cutting open the fish, check for blood or bile coming out of the anal pore which might suggest an intestinal injury.

Internal Signs of Barotrauma

A ventral cut from the anal pore up to the pericardial region will expose all internal organs of interest. When cutting, it is important to cut through the ventral musculature but not too deep that organs are damaged by the scissors. Insert the ball-point into the vent to begin the incision. When cutting, apply a slight outward pressure with the ball point on the abdominal wall to provide a visual cue for cutting to help keep from cutting too deep. There will be some pressure when cutting through the clavicle of the pericardium. If there is any concern of cutting into the heart then stop cutting before the pericardial region. Carefully pull apart the remaining tissue with forceps on either side of the cut.

7. Upon exposing the internal organs, it is important to initially look for any signs of blood that would suggest an internal injury. Likely sources of clotted blood include the intestine, kidney, liver, and heart.
8. **Liver** – Look for hemorrhaging on or around the liver and check the hepatic duct for perforation; to see this lift the liver and look underneath for damage.

9. **Heart** – The heart can be exposed by carefully pulling apart both the left and right sides of the body cavity with forceps thus tearing the pericardial sac. Look for hematoma on the heart, hemorrhaging from the heart, or embolisms.

10. **Spleen** – Look for signs of hemorrhage.

11. **Fat** – Look for signs of hematoma.

12. **Gall Bladder** – Look for signs of damage that would display as discoloration of internal fluid such as red or brown instead of bright green

13. **Pyloric Caecum** – Look for signs of damage and or condition, if over feeding the ceaca will be extra thick.

14. **Intestine** – Inspect the entire intestine for food and amount of food; only allow less than half inch of food in the gut. Inspect the intestinal veins for damage.

15. **Gonads** – Examine testes or ovaries for any bruising or hemorrhaging. (Gonads appear seasonally and depend upon age of the Chinook salmon smolts).

16. **Swim bladder** – Move all the guts to the side: intestine, stomach, and any fat, to expose the entire swim bladder. A typical swim bladder is surrounded by a thin membrane which needs to be gently removed to expose the swim bladder. A perforation of the swim bladder can be difficult to detect because the overlying membrane can trap air making the swim bladder appear inflated. Close examination of the swim bladder can usually reveal the location of a perforation.

17. **Kidney** – Removal of the swim bladder reveals the kidney, which lies along the dorsal surface of the abdominal cavity. Examine the entire length of the kidney, from most anterior (under the heart) to most posterior (to the vent) for the presence of any bubbles or hemorrhaging under the surface; and for damage to the vessels along the muscle walls.

18. **Otoliths** – Approaching from the ventral side, remove the lower jaw and gills. Enter through the roof of the mouth and carefully remove soft tissue and cartilage to expose caudal part of ear (saccule). Remove both saccular otoliths (left and right sides) and note the crystalline structure of aragonite (white) or vaterite (clear).

19. **Store Otoliths** – Store both otoliths in a vial. Label the vial; include

    a. 1) Your initials (person who did necropsy)
    b. 2) AA, AV, or VV (for the aragonite/vaterite combination)
    c. 3) Fish ID
    d. 4) Date (mm/dd/yyyy)
    e. 5) Salmon (if not part of fish ID)

Copy of Shipping Permit

MARYLAND
DEPARTMENT OF
NATURAL RESOURCES

Martin O'Malley, Governor
John R. Griffin, Secretary

July 21, 2009

Dr. Arthur Popper
Dr. Michele Halvorsen
Department of Biology
The University of Maryland
College Park, MD 20740

Dear Sir and Madam:

I have been asked to provide guidance and permission for your laboratory at The University of Maryland to import chinook salmon fingerlings from the Pacific Northwest National Laboratory in Richland, Washington. The shipments would take place in 2009 and 2010, and total approximately 1200 fingerlings per year.

Under the authority of Natural Resources Articles 4-602 and 4-11A-02, which regulate the possession and use of non-native fish, permission is granted to your laboratory to import and possess the fish under the following requirements. Once received, the fish must remain within the laboratory and under the guidance of the UM Attending Veterinarian working on behalf of your Institutional Animal Care and Use Committee (IACUC). No releases are allowed into Maryland waters, and there should be measures in place to avoid the release of any fish, pathogens or gametes from your laboratory. At the conclusion of the experiments, all remaining surving fish must be disposed of in a sanitary manner as described in the IACUC protocols.

If there are any questions, please contact me at rbohn@dnr.state.md.us or by phone at 410-260-8317.

Sincerely,

Richard Bohn

Richard Bohn
Permit Coordinator, Fisheries Service
Maryland Department of Natural Resources

cc: Harley Speir
Brandon Casper

Tawes State Office Building • 580 Taylor Avenue • Annapolis, Maryland 21401
410.260.8DNR or toll free in Maryland 877.620.8DNR • www.dnr.maryland.gov • TTY users call via Maryland Relay

APPENDIX E

STATISTICAL ANALYSIS

Assessment of Fish Response Severity Index (RSI) and Exposure to Acoustic Pressures Simulating Pile Driving

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24 February 2011

Study Objective

To assess the relationship between fish conditions as measured by a severity index (RSI) and exposure to different acoustic pressure scenarios defined by single strike strength (SEL_{ss}) and number of strikes reminiscent of pile driving in aquatic environments.

Background

The effects of exposure to acoustic pressures reminiscent of pile driving on Chinook salmon smolts were examined. Exposure levels were measured in terms of SEL_{ss}, number of strikes (960 or 1920), and $SEL_{cum} = SEL_{ss} + 10 \log_{10}$ (number of strikes). The response variable was the fish severity index (RSI) measured at the individual level. Individual fish were exposed to 1 of 11 different treatment combinations of SEL_{ss} and number of strikes. The 1st treatment was performed at 1920 strikes and at an SEL_{ss} setting to produce the highest value of SEL_{cum} tested (Table 1). The following ten treatment combinations were coordinated in a balanced design resulting in five different levels of SEL_{cum} (Table 1). The number of strikes was set at either 960 or 1920, and SEL_{ss} levels adjusted to provide five different values of SEL_{cum}. Fish were exposed to the acoustic treatments one at a time and exact exposure levels varied from the selected target values of SEL_{ss} and SEL_{cum} in Table 1.

Table 1: Summary of 11 different test conditions defined by single strike strength (SEL_{ss}), number of strikes (960 or 1920), and SEL_{cum}, along with the number of fish tested and resulting average value of the fish response severity index (RSI).

Treatment	*N*	Average SEL_{ss}	No. of Strikes	Average SEL_{cum}	Average RSI
1	44	187	1920	219	15.318
2	35	183	1920	216	5.971
3	28	186	960	216	6.071
4	26	180	1920	213	2.346
5	31	183	960	213	4.323
9	30	177	1920	210	3.433
8	31	181	960	210	4.032
7	43	174	1920	207	0.581
6	24	177	960	207	1.042
11	31	171	1920	204	0.419
10	32	174	960	204	0.656

Statistical Methods

The response variable RSI was transformed before analyzed to

$$y_i = \ln(\mathrm{RSI}_i + 1)$$

to stabilize variance and linearize the response curve. Cumulative energy was expressed as

$$\mathrm{SEL}_{cum} = \mathrm{SEL}_{ss} + 10\log_{10}(\text{number of strikes}).$$

Analyses of covariance (ANOCOV) was performed regressing y_i against SEL_{cum} and assessing whether number of strikes (960 or 1920) had an additional effect on fish response beyond that described by SEL_{cum}. Initial analyses were conducted on the balanced design using treatments 2-11 in Table 1. Analyzing all 11 treatment combinations would have confounded treatment comparisons with range of SEL_{cum}. Once a model was selected using the balanced design, treatment 1 was added to the analysis to refine the results.

Analyses were also performed on the separate treatment combination with different number of strikes but similar SEL_{cum} levels using once again ANOCOV. The test-wise α-levels were adjusted for an experimental-wise α-level of 0.05 at $\alpha_{TW} = 0.0102$.

A contour plot was constructed graphing SEL_{cum} as a function of the number of strikes and SEL_{ss}. Superimposed on this contour plot were contours of predicted values of RSI.

Results

The ANOCOV found that $\ln(\mathrm{RSI}+1)$ was linearly related to SEL_{cum}, and fish exposed to a common value of SEL_{cum} by different numbers of strikes (i.e., 960 or 1920) had different RSI values. Using treatments 2-11 in the balanced design, common slopes were found for the regression of $\ln(\mathrm{RSI}+1)$ vs. SEL_{cum}, but different intercepts for fish exposed to either 960 or 1920 strikes (Table 2). Fish with 960 strikes had a significantly higher $\ln(\mathrm{RSI}+1)$ value than fish exposed to 1920 strikes at the same value of SEL_{cum}. In other words, for common values of SEL_{cum}, higher values of SEL_{ss} resulted in significantly higher values of RSI. Adding treatment 1 (i.e., $\mathrm{SEL}_{ss} = 186.6$, number of strikes = 1920) to the analysis did not change the linearity of the data on the ln-scale or the regression relationships (Table 3, Figure 1). The final model was based on the use of all observations.

Table 2: Sequential analysis of covariance (ANOCOV) results for treatments 2–11. Response: $\ln(\text{RSI}+1)$. Best model contains the covariate SEL_{cum} followed by strikes.

Treatments 2–11

Source	df	SS	MS	*F*-value	*P*-value
SEL_{cum}	1	103.22	103.22	281.240	<0.0001
Residuals	309	113.41	0.37		

Source	df	SS	MS	*F*-value	*P*-value
SEL_{cum}	1	103.22	103.22	289.679	<0.0001
Strikes	1	3.95	3.95	11.077	0.0010
Interaction	1	0.07	0.07	0.196	0.6583
Residuals	307	109.39	0.36		

Best Model

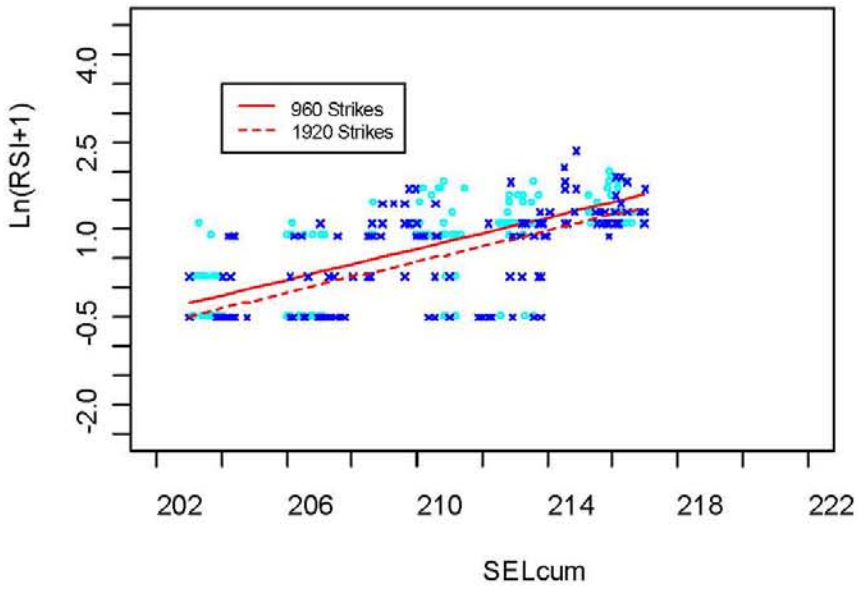
Source	df	SS	MS	*F*-value	*P*-value
SEL_{cum}	1	103.22	103.22	290.437	<0.0001
Strikes	1	3.95	3.95	11.106	0.0010
Residuals	308	109.46	0.36		

Table 3: Analysis of covariance (ANOCOV) from the best model from Table 2 applied to all treatments 1–11. Response: $\ln(\text{RSI}+1)$.

All Treatments

Source	df	SS	MS	*F*-value	*P*-value
SEL_{cum}	1	201.30	201.30	542.039	<0.0001
Strikes	1	2.24	2.24	6.033	0.0145
Residuals	352	130.72	0.37		

a. Scatterplot for treatments 2–11



b. Scatterplot for treatments 1–11

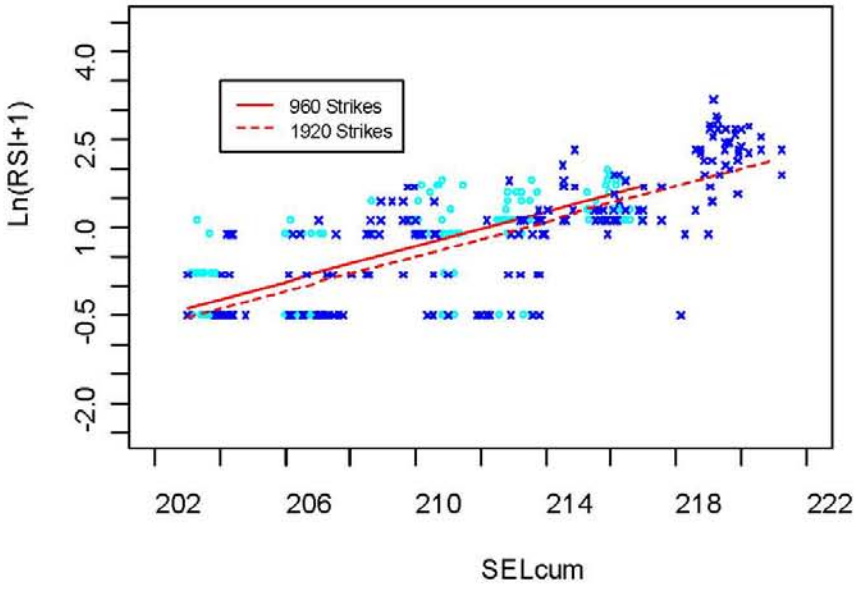


Figure 1: Scatterplots of SEL_{cum} vs. $\ln(RSI+1)$ (a) for treatments 2–11 and (b) all treatments. Red lines show predicted $\ln(RSI+1)$ values for 960 and 1920 strikes. An 0 denotes a 960-strike trial and an x denotes a 1920-strike trial.

The ANOCOV on the individual pairs of treatments (i.e., 2 & 3, 4 & 5, 6 & 7, 8 & 9, 10 & 11) was based on the same general form of the model as was used on all the data (Table 4). These ANOCOV found only treatments 4 & 5 to be significantly different at an experimental-wise α-level of 0.05 (test-wise α-level of 0.0102). Scatterplots of the pairwise comparisons readily illustrate no apparent differences with considerable overlap of the 960 and 1920 strike trials in RSI values (Figure 2). These individual tests are much less powerful than the full analysis (Table 3).

A contour plot of RSI illustrates values increase as SEL_{ss} increase (Figure 3). It is important to note that the linear contours in Figure 4 are the result of testing at only two levels of strike numbers. It is unknown whether that linearity would persist if additional levels of strike numbers were tested.

Discussion

There was a highly significant relationship between RSI and SEL_{cum} ($P < 0.0001$). That relationship is linear on the log-scale (Figure 1). However, the relationship is modified by the particular combination of SEL_{cum} and strike numbers contributing to the overall value of SEL_{cum}. All else being equal, higher values of SEL_{ss} and lower strike numbers produce a higher RSI.

Table 4: Pairwise comparisons between treatments with similar SEL_{cum} values.

Treatments 2 vs. 3

Source	df	SS	MS	*F*-value	*P*-value
SEL_{cum}	1	0.15	0.15	1.517	0.2230
Strikes	1	0.05	0.05	0.471	0.4954
Residuals	60	6.12	0.10		

Treatments 4 vs. 5

Source	df	SS	MS	*F*-value	*P*-value
SEL_{cum}	1	0.75	0.75	1.625	0.2079
Strikes	1	5.16	5.16	11.188	0.0015
Residuals	54	24.89	0.46		

Treatments 6 vs. 7

Source	df	SS	MS	*F*-value	*P*-value
SEL_{cum}	1	0.01	0.01	0.046	0.8317
Strikes	1	0.54	0.54	1.659	0.2024
Residuals	64	20.98	0.33		

Treatments 8 vs. 9

Source	df	SS	MS	*F*-value	*P*-value
SEL_{cum}	1	1.05	1.05	2.926	0.0925
Strikes	1	0.92	0.92	2.585	0.1133
Residuals	58	20.72	0.36		

Treatments 10 vs. 11

Source	df	SS	MS	*F*-value	*P*-value
SELcum	1	0.24	0.24	1.097	0.2992
Strikes	1	0.15	0.15	0.677	0.4138
Residuals	60	13.32	0.22		

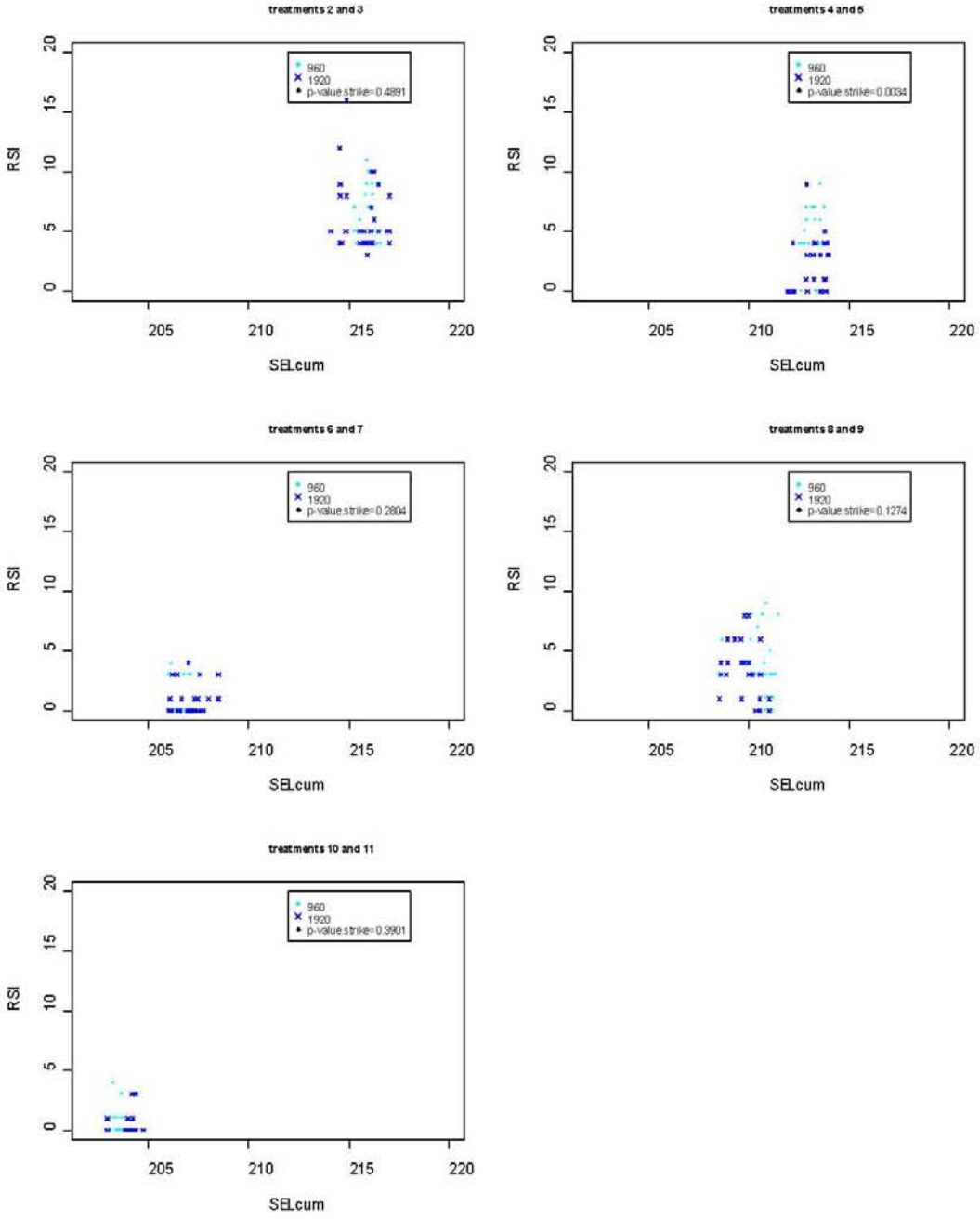


Figure 3: SEL_{cum} vs. RSI plots grouped by treatments with similar SEL_{cum} values. Each plot shows the *P*-value for the difference between strike groups for pairwise comparisons between treatments.

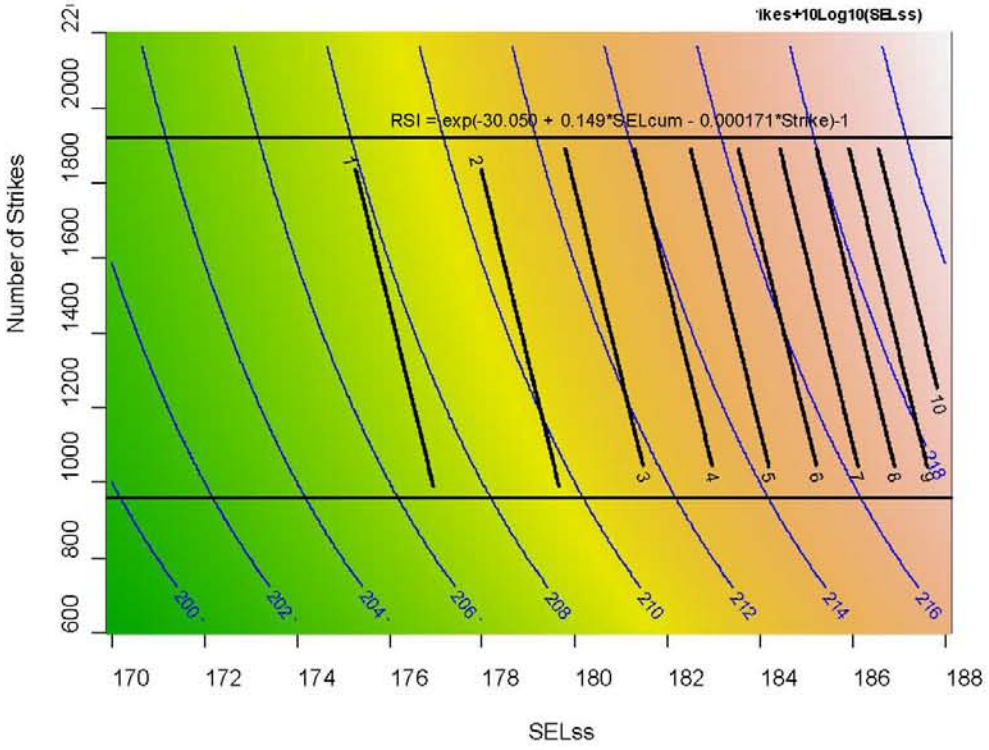


Figure 4: Contour plot of SEL_{cum} with predicted RSI scores superimposed.

APPENDIX F

FISH MAINTENANCE

Study Fish

This study used juvenile Chinook salmon. The average fish was 103 ± 8.75 SD mm standard length and average weight was 11.8 ± 3.47 SD g (Figure F-1).

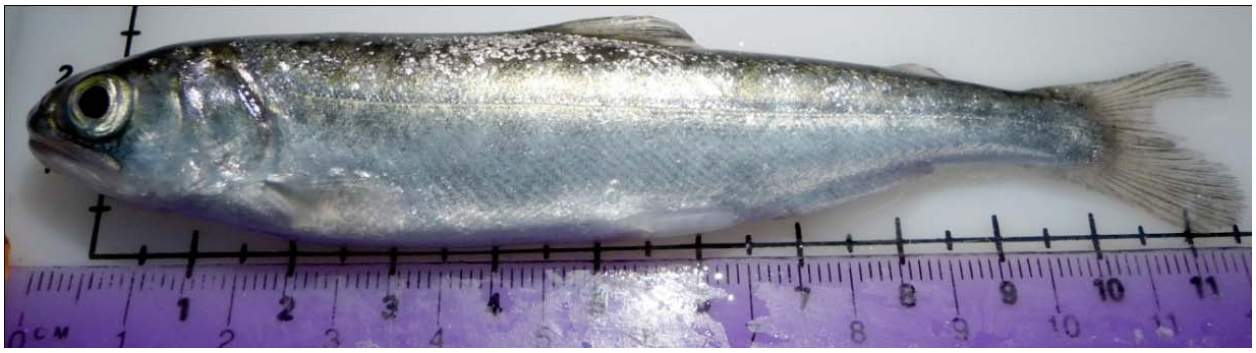


Figure F-1. Juvenile Chinook salmon used in this study. Note: Caudal fin clipped for identification purposes.

The Chinook salmon were provided by Pacific Northwest National Laboratory from the Priest Rapids Hatchery in Mattawa, Washington. They were shipped via FedEx overnight from Richland, Washington, to Baltimore, Maryland, under authority of the Maryland Department of Natural Resources (Natural Resources Articles 4-602 and 4-11A-02). Fish were picked up from the FedEx distribution facility in Linthicum, Maryland, and driven to the University of Maryland, College Park. Total travel time was approximately 20 hours. The fish were shipped in large plastic bags containing 11.4–18.9 L of conditioned, oxygenated water. Each bag contained 30–50 fish. The bags were placed in insulated coolers with ice packs to hold water temperature below 10°C.

Fish Maintenance

Once at the university, the sealed bags were floated in a holding tank (Tank 1 in Figure F-2) containing 10°C water for one to two hours to slowly bring the fish to the tank temperature. The bags were then opened and fish were caught individually using small nets, inspected for health, and placed into the holding tank. All dead or seemingly unhealthy fish were not placed in the holding tank. The water from the shipping bags was never mixed with the tank water. The fish acclimated in the holding tank for a minimum of two weeks before they were used in an experiment.

Fish were kept in a dedicated aquarium room of the laboratory (Figure F-2) in the Biology/ Psychology building at the University of Maryland. This room met all federal standards for animal care. The care and maintenance of the room, as well as the conduct of all experiments described in this report, were done under protocols reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Maryland (see Appendix D). Fish were held under authority of the Maryland Department of Natural Resources (Natural Resources Articles 4-602 and 4-11A-02). Details of fish maintenance are discussed in Appendix G.

The room was lit with fluorescent bulbs and controlled by a timer on a 24-hour light:dark cycle—10 hours light:14 hours dark. Midway through the study the bulbs were changed from 4100 K to more energy-efficient 5000 K bulbs but then changed back 4100 K after 6 months because of algae growth issues that resulted in fish eating uncontrolled amounts of food. The room was electrically protected with a backup power system.

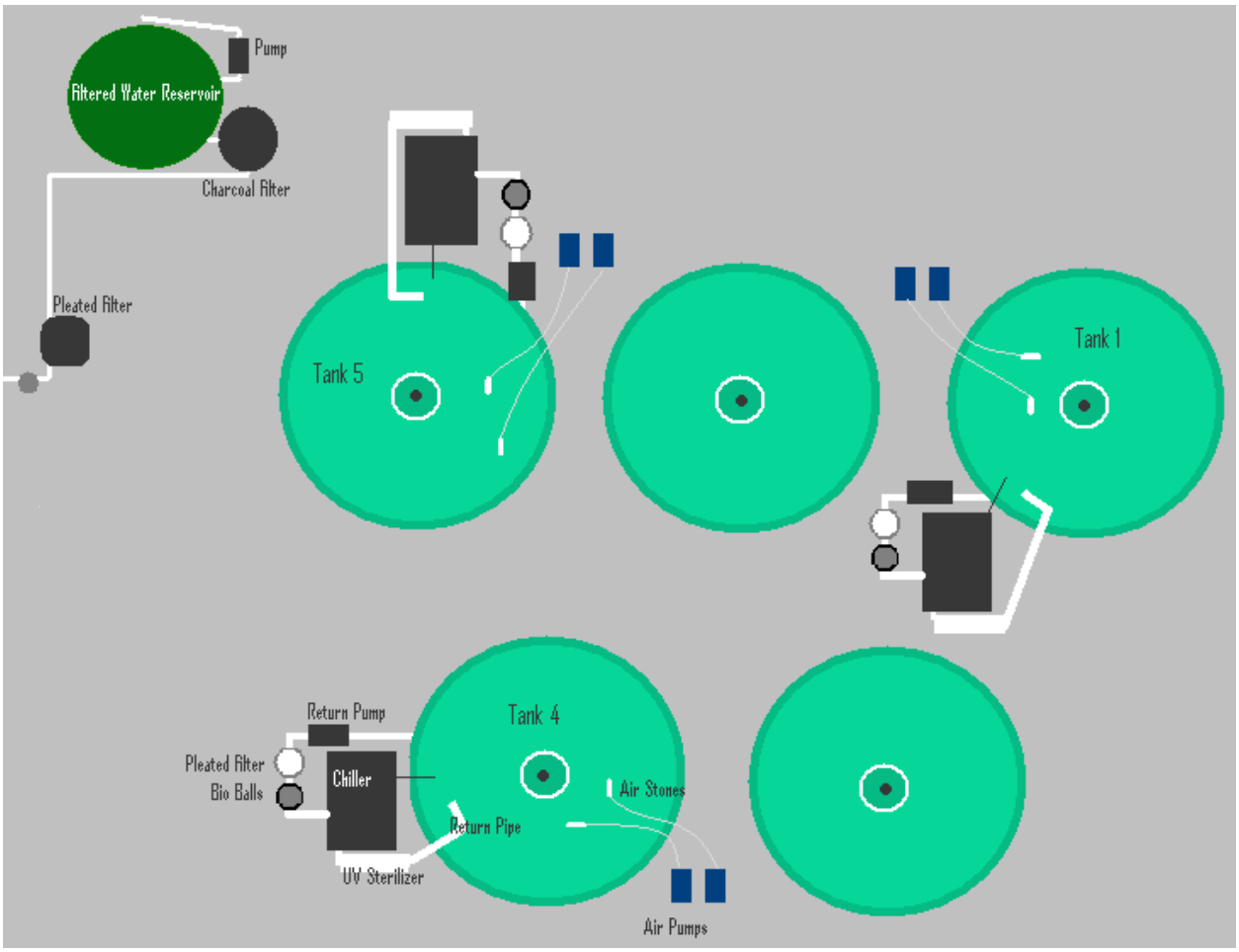


Figure F-2. Setup of the fish room in which the Chinook salmon were maintained. Each fiberglass cylindrical tank used in the study (1, 4, and 5) was 1.22 m in diameter and 0.76 m high. Water depth was kept at approximately 0.69 m, resulting in an approximate volume of 802 L. Water temperature was monitored constantly.

Each tank was a closed system. Water flowed through a center drain to a Little Giant pump (4-MDQ-SC; 2895.8 L/hr). The water was pumped through a microfiber pleated filter, followed by bio-balls, and then through a chiller (Delta Star titanium in-line water chiller D5 ±1°C). Finally, the water was passed through an ultraviolet sterilizer before being returned to the tank.

Flow was directed toward the side of the tank to provide a circular current that was strongest near the surface of the water and weaker towards the bottom, providing the fish with a current to swim against as well as calm areas. Aeration was provided by two Aquatic Ecosystems Sweetwater SL24 air pumps that fed porcelain air stones.

Fish went through a temperature acclimation process prior to being used in experiments. The fish in Tank 1 (holding tank) were the stock population and were maintained at 10°C to control for fish size and growth. Following at least two weeks initial acclimation in Tank 1, fish were moved to Tank 5 where they were acclimated to 12°C for at least one week. Fish were then clipped for identification (as shown in Figure F-1), transferred to Tank 4, and held at 14°C for at least 2.5 days prior to use in experiments.

The fish were fed BioOregon Grower Diet. Each of the three tanks had a different feeding regimen. Fish in Tank 1 were fed every Monday, Wednesday, and Friday. The quantity of food varied based on the number of fish in the tank. The fish were fed enough so that all fish were able to eat. Excess food was removed from the bottom of the tank. Fish in Tank 5 were fed only on Monday and Wednesday. Fish in Tank 4 were not fed to ensure that they did not have food in their gut during the experiment. However, any fish remaining in Tank 4 at the end of the week were fed enough so as to hold them over until the following week. During feeding, air pumps and filtration were turned off and the fish were observed while eating.

Each tank underwent a strict water change regimen using conditioned water (originally from the public supply) with chlorine and other chemicals removed. Stock Tank 1, with the largest biological load, had a 50% water change twice a week. Tank 5 received one 30% and one 50% water change per week. Tank 4, which was fed infrequently, had a much lighter biological load than the other two and received one 50% water change per week. Each tank was equipped with a drain that was used to remove water during the water changes. Replacement water was filtered by a Shurlok Jacuzzi pleated filter and a pressurized Hayward S200 high-rate sand filter filled with charcoal. The filtered water was held in a 624.59-L aerated reservoir. Filtered water was tested for chlorine, and pH levels were monitored on a weekly basis.

Water quality testing of the tanks consisted of nitrate and nitrite testing using test strips, pH testing using an electronic pH meter, and chlorine testing using test strips. Testing was done every Monday, Wednesday, Friday, and any time fish were behaving abnormally or when a dead fish was found. Temperature was checked every day on each tank to ensure that the chillers were maintaining proper water temperatures.

Health Maintenance

Fish were observed every day for any signs of stress or illness, included darkening in color, behavioral scratching of the bottom of the tank, gaping mouth, lying on the tank bottom or against the tank walls, or showing visible physical injury. Fish were also checked for signs of illness each time they were moved from one tank to another to prevent contamination into the experimental population. Fish that had pop-eye or any type of growth on their body or that were scratching constantly on the bottom or sides of the tank were removed and euthanized. Fish that had darkened color were left in the tank and observed more carefully for signs of emerging illness over several days. If a large portion of the fish in a tank were showing dark coloration, salt was added to the water up to 10 ppm to help fight off potential infection. Fish euthanasia was accomplished using a buffered solution of tricaine methanesulfonate (MS-222) with a concentration of 250 mg/L in fish Ringers solution.

Tail Clipping for Fish Identification

Fish that had been in Tank 5 for at least one week were clipped to make them easier to identify during an experiment. This was done by anesthetizing fish lightly with buffered MS-222 concentration of 40 mg/L and then using a hole punch dipped in Stresscoat to clip off a small portion of the caudal fin in one of four ways: dorsal, ventral, double (both dorsal and ventral), or no clip. Fish that received no clip still went through a sham clipping process to ensure consistency. After being clipped, each fish was immediately placed into a recovery bucket containing tank water, Stresscoat, and an air stone until normal swimming resumed.

For each experiment, two fish of with different clip patterns were caught, placed in an insulated bucket containing tank water and covered with a lid, and taken to the sound exposure apparatus for

experimentation. The experimenters took extra caution to reduce stress and not scare the fish while capturing them for the experiment since fish under stress will expel air from their swim bladders and become negatively buoyant and unusable in the experiment. Instead of chasing the fish with the nets, the experimenters corralled the fish with slow movements of the nets.

APPENDIX G

SOUND APPARATUS (HICI-FT) AND CONTROL

GENERAL EXPERIMENTAL PROCEDURES

Sound Exposure Apparatus and Methods

Sound exposure was conducted in a system called the High Intensity Controlled Impedance Fluid-filled wave Tube (HICI-FT). The HICI-FT is a specially designed wave tube that uses large shakers to produce sounds that accurately reproduce actual pile driving sounds. In the HICI-FT, fish are exposed to sounds with specific acoustic signatures. Moreover, the number, duration, and other aspects of the pile strikes could be controlled. Thus, the HICI-FT enabled the investigators to provide the first quantified data on effects of pile driving signals on fish physiology.

The HICI-FT was designed and constructed at Georgia Institute of Technology by Dr. Peter Rogers and Mr. Jim Martin. Mr. Martin did the construction and wrote the software to control the HICI-FT and sounds associated with it.

The HICI-FT enabled presentation of actual pile driving sounds in the laboratory and allowed for control of the parameters that affect pile driving signals. Thus, the parameters adjusted enabled presentation of stimuli at different cumulative sound levels, single strike level, and number of strikes, along with playing eight different pile driving signals. The sound presentation was controlled using LabVIEW (National Instruments Corporation, Austin, Texas) and the stimuli as played in the HICI-FT during experiments were captured with a hydrophone and digitized by LabVIEW. In addition, Dazzle MovieStar software (http://dazzle-moviestar.software.informer.com/) captured digital images of a 45° region inside the HICI-FT during experiments. Digital images allowed the observer an occasional glimpse of a fish if it swam into the angle of view. (Note, these observations were primarily to check on fish survival and cannot be used for behavioral studies.)

The HICI-FT chamber (Figure G-1) was a circular tube 0.45 m long with a 0.25-m internal diameter and 3.81-cm-thick stainless steel walls filled with water. At either end of the tube was a rigid lightweight circular piston held by a membranous seal in the center of the steel end cap. Each piston was connected to a linear electrodynamic motor (moving coil shaker) anchored to the end caps. The motors of the shakers were driven separately with signals appropriate to create the desired pressure and velocity fields within the tube for the sound exposure of the fish.

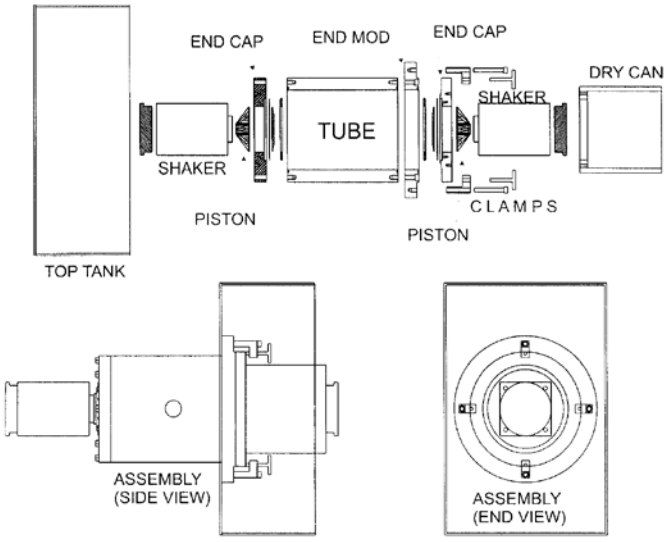


Figure G-1. The HICI-FT as described in the text. The section labeled top tank is the acrylic water-filled chamber in which the fish were placed during exposure. The HICI-FT is shown in the horizontal position used during sound exposure.

The inner chamber of the HICI-FT is shown in Figure G-2. During operation, the chamber housed fish as well as a hydrophone to record the signal, a dual light source, and a digital camera. The device was designed so that the central axis of the cylindrical chamber would be vertical when fish were placed inside. The tube then was rotated into the horizontal orientation to present sound in a natural orientation with respect to the acoustic velocity vector of the incident sound field from pile driving. The manipulation of the chamber was accomplished by mounting in a support buggy that allowed rotation of the chamber around its center of gravity (Figures G-3 and G-4). Rotation of the device is shown in Figure G-5.

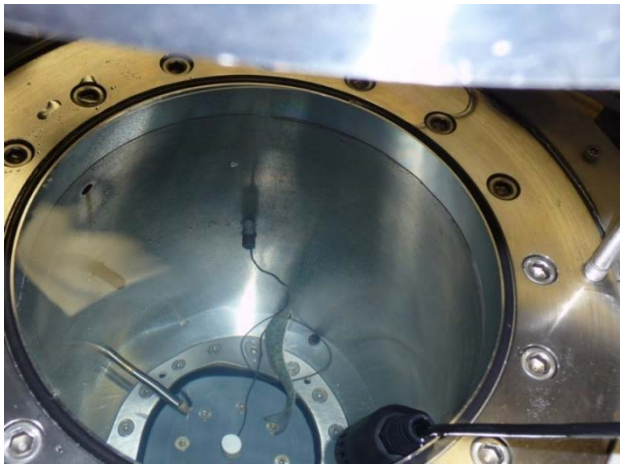


Figure G-2. View inside the HICI-FT chamber (vertical position). A Chinook salmon can be seen toward the bottom of the tube. The long silver cylinder on the left is the light source and digital camera. Just above this on the left is the hydrophone (black, smaller tube). The wire in the center is for an accelerometer (white cylinder attached to the bottom of the tube). The large black device (lower right) measured temperature and total dissolved gas of the water in the tube after each experiment. The bottom of the tube shows the face plate for the piston coupled to the lower shaker.

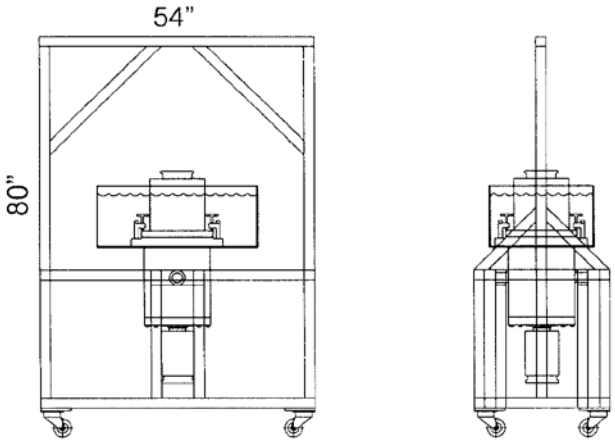


Figure G-3. HICI-FT support buggy enabling device rotation for experiments. In this image, the HICI-FT chamber is closed and the device is in the vertical position. The rectangular chamber (left) was where the fish was placed initially for acclimation to the chamber water. During operation, the wheels of the buggy were lifted off the floor by vibration isolation devices that uncoupled the buggy from the building floor.

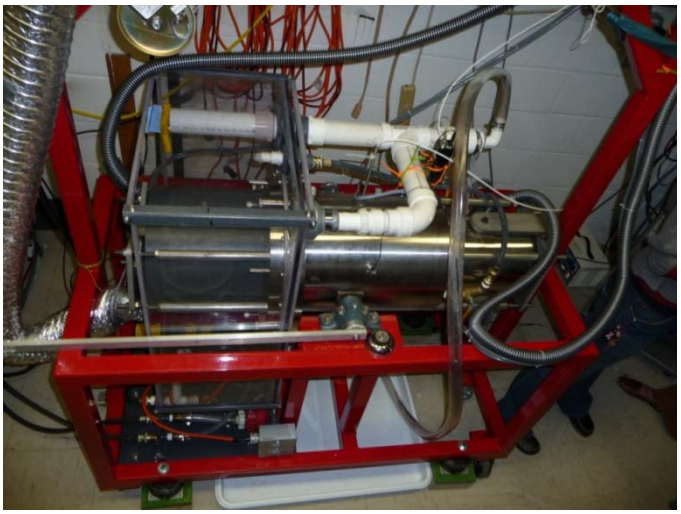


Figure G-4. The HICI-FT in the horizontal position with the top part (if in the vertical position) to the left. The red structure is the buggy supporting the HICI-FT. The white polyvinyl chloride pipe is part of the water drainage system. The flexible grey hoses are part of the system for cooling the shakers.

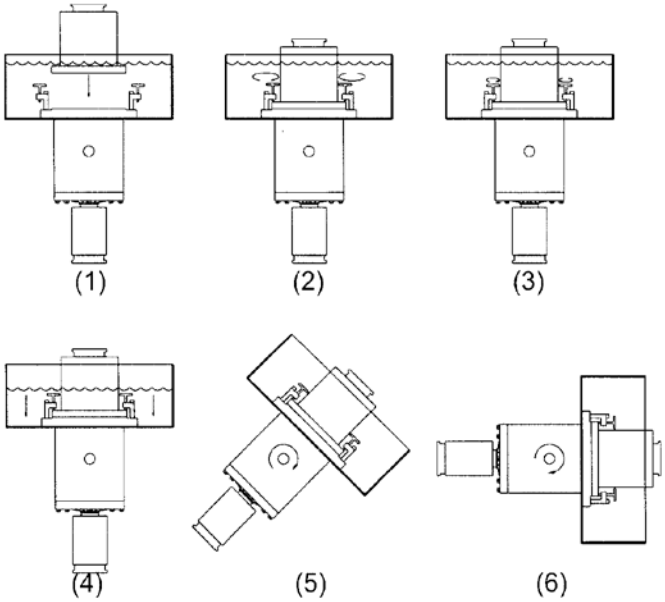


Figure G-5. Method to place fish into the HICI-FT and rotate it to the horizontal position. In (1), the top shaker is open and the chamber is exposed. Each fish was placed into the acrylic acclimation box (rectangle) for 20 min. After 20 min, a mesh cover over the opening to the chamber was removed and the fish swam down into the HICI-FT tube. The top shaker was then lowered into place (2) and clamps tightened (3) so that the top shaker was firmly mated to the rest of the HICI-FT (this was done by a pulley system that allowed slow lowering of the shaker into place). Water was then drained from the top tank (4), and the HICI-FT was then rotated (5) to the testing position (6). Removing fish from the tube involved reversing these procedures.

The HICI-FT and its design offer several key features. The thick steel walls of the device are very rigid an enabled achieving a plane-wave stimulus similar to that found in a far-field of a sound source with a sound speed and impedance that were close, if not identical, to those of open water. Moreover, the

presence of pistons at opposite ends of the chamber allowed them to be driven in or out of phase with one another, providing the ability to modify the pressure and particle motion fields in the chamber and generate a plane-wave.

Because the entire HICI-FT system produced a considerable heat that could cause system failure, aggressive action was taken to cool the room and the shakers. The output of the room air condition vent was fed, with a hose, directly into the top shaker armature casing. The cooling blower motors associated with each shaker were mechanically modified to work more efficiently thereby removing more heat. Finally, the exhaust from the blowers was released directly into a high-velocity ceiling-vent designed for fume-hood evacuation. The ceiling vent directly removed the shaker exhaust heat and gave the room a high air exchange rate.

Sounds

The HICI-FT chamber was designed to produce propagating plane waves with a peak sound pressure level (SPL) of at least 215 dB re 1 µPa. The HICI-FT was able to generate pressure and particle motion levels that were very similar to those produced by pile driving activity.

The pile driving signals used in this study were analogues of field recordings of both pressure and particle motion taken at a range of 10 m from a steel shell pile driven using a diesel hammer at the Eagle Harbor Maintenance Facility (MacGillivray and Racca, 2005). The actual sound exposure paradigms were designed to mimic actual pile driving activities. Thus, the experimental characteristics of each sound exposure matched real-life activity, such as the time and frequency domain characteristics of each pile strike, inter-strike-interval, and number of strikes.

The signals used in the experiments consisted of eight different pile driving strikes, which were normalized to the same SEL and compiled into a single file that contained exactly 12 repetitions of each of the 8 signals, for a total of 96 strikes. MATLAB (The MathWorks, Inc., Natick, Massachusetts) was used each day to generate a randomization of the 96-strike file. This file then was used by LabVIEW for the day and repeated 10 times for a 960-strike presentation or 20 times for a 1920-strike presentation. Therefore, fish receive a pseudorandom presentation of pile strikes, and every day was different.

Figure G-6 shows the time domain and power spectral density of two of the eight strikes. The rationale for having eight different strikes was the inherent variability among pile strikes due to force of the hammer strike, substrate, depth of the pile within the substrate, and other factors. Thus, using several different strikes within the sound presentation more closely replicated a realistic pile driving scenario.

HICI-FT Preparation

The water used to fill the HICI-FT was conditioned for use in the experiment. This required removing all air bubbles from the water because they could affect sound transmission characteristics and therefore alter the sound signal to which the fish were exposed.

Water from the building supply system was passed through a charcoal filter as well as a smaller filter to remove any debris from the water before conditioning. The water conditioning assembly consisted of a reservoir connected to filtration and chilling equipment. A pump pulled water from the bottom of the reservoir, which then passed through a charcoal and pleated filter, through a chiller set at 12°C, and finally was released over a container of bio-balls before being returned into the reservoir. The bio-balls removed excess gases from the water to maintain the water as close as possible to 100% total dissolved gas. This step was essential because the water originating from the building was often supersaturated

with gas. The water temperature was held at 12°C because after the HICI-FT was filled and the final preparations were complete, the temperature increased to about 13°C, a desirable temperature at which to begin experiments.

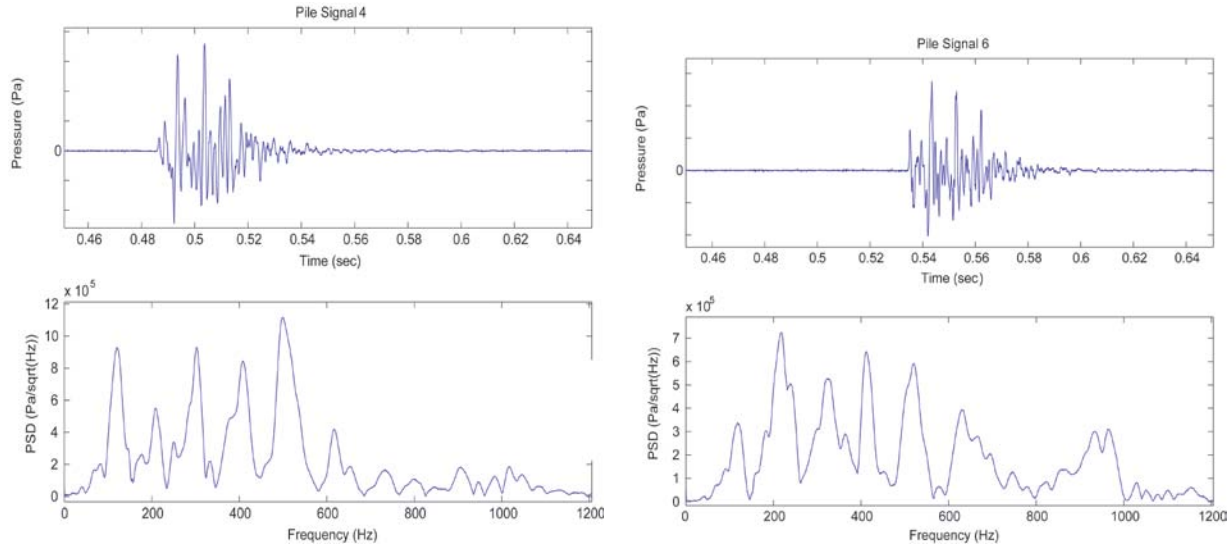


Figure G-6. Two of the eight signals used in the study. In each figure pair, the upper image shows the time-domain of the signal while the lower panel shows the power spectral density.

Before the HICI-FT was filled, the drain was plugged with a drain trap and plug. A pump was used to completely fill the steel tube of the HICI-FT with conditioned chilled water. Following this, a water dispenser, filled with conditioned water, was used to force water into all holes, seals, and anywhere else that air bubbles might be during the filling of the tube. This was necessary in order to manage the compliancy of the water in the tube, a necessary step to achieve a predictable and consistent sound field. After the bubbles were removed, the acclimation box was filled to two-thirds of its depth with conditioned water. Bubbles were then removed with the water dispenser in all newly submerged portions of the tube, including the O-ring. The top shaker was then lowered into the water with a winch and air bubbles caught on the shaker face were removed with the water dispenser. The top shaker was then completely lowered onto the steel tube. A hose delivering cool air from the air-conditioning vent was then fitted into the top shaker and the four vibration isolation mounts were filled with air to decouple the HICI-FT from the building floor. Finally, the two cooling blowers were turned on, and the system was warmed up for 15 min.

HICI-FT Sound Control Operation

Signal generation and data acquisition for the HICI-FT was handled with a Dell laptop computer and a 12-bit analog/digital (A/D and D/A) converter (National Instruments Corporation, Model PCI-MI0 16E1). Software code (proprietary to the HICI-FT designer, James Martin) was written in LabVIEW and compiled for use in the system. Analog drive signals were generated on two separate channels of the A/D converter. These were filtered and attenuated using anti-aliasing filters and programmable attenuators (Tucker-Davis Technologies [TDT], Alachua, Florida, Model PA4). The attenuators provided the system with 100 dB of dynamic range beyond the 12-bit resolution of the A/D converter. The attenuators were controlled through a serial interface with the PC. The outputs of the attenuators were amplified by an amplifier (Crown International, Elkhart, Indiana, Model XTi 4000), one to each shaker (Vibration Test Systems, VG-150 Vibration Generator, Model VTS 150).

Four channels of the A/D interface were used for data acquisition at a sampling rate of 10 kHz. Two channels were used as drive system monitors, and the other two were used to monitor the acoustic field inside the tube. The acoustic field monitor channels were equipped with low-noise preamplifiers (Stanford Research Systems, Inc. [SRS], Sunnyvale, California, Model 560). One was connected to a calibrated hydrophone (Brüel & Kjær Sound & Vibration Measurement A/S, Naerum, Denmark, Model 8103) and the other could be connected to an accelerometer (MISTRAS Group, Inc., Princeton Junction, New Jersey, Vibra-Metrics Model 9002) encapsulated in syntactic foam for neutral buoyancy.

The output of the hydrophone was amplified by an SRS Model 560 preamplifier and then fed into the A/D board. The signal also was fed into a digital recorder (Marantz America, Inc., Mahwah, New Jersey, Model PMD-671) for full digital archival of each exposure because the signals as fed directly to the computer were recorded only partially (due to their size). Furthermore, although LabVIEW could use this signal to ensure that the sound was correct, it was not able to store the signal. After the experiments were completed, the SEL_{cum} for each exposure was reconfirmed for the entire signal recorded on the Marantz recorder.

All information regarding each experiment was recorded on data sheets. An example of the data sheet is in Appendix C.

General Experimental Procedures

At the start of each day the HICI-FT was filled with water and the SEL_{cum} level was set for the day using an eight-signal file. After SEL level determination, fish acclimation began. The temperature had to be at least 13°C (but not more than 14°C) before fish were placed in the water. Two Chinook salmon were carefully transferred with a mesh net from the transportation buckets into the acrylic acclimation chamber (Figure G-5). The transportation bucket was kept on ice to keep the water at 14°C.

The fish were allowed to swim freely in the chamber for a 20-min acclimation period. During this time, they were observed, with specific focus on the actions of gulping air, burping air, and the swimming attitude (buoyancy). After the 20 min, the fish were scored to be negatively, neutrally, or positively buoyant, which was determined from the body position of the fish while swimming within the acrylic acclimation chamber. If the fish was swimming horizontally (parallel to the bottom of the chamber) with smooth motions it was classified as being neutrally buoyant. If the fish was swimming with its anterior portion angled downward and/or appeared to be struggling to swim, or if it just sat on the bottom of the box for the duration of the acclimation period, the fish was classified as negatively buoyant. If the fish was swimming with its anterior portion angled toward the water surface and appeared to be struggling to swim, it was classified as positively buoyant.

Following the acclimation period, the plastic mesh covering the HICI-FT tube was slowly removed so as to not alarm the fish and cause them to burp out gas from their swim bladder and to allow access into the tube. If a fish did not enter the tube, a net was carefully used to corral it into the tube. The top shaker was then lowered onto the tube with a winch and pulley. Any bubbles that had formed on the submerged shaker face were removed before complete closure of the tube. The shoulder bolts were then tightened to seal the tube and all water was drained out of the acclimation chamber. The HICI-FT was rotated from the horizontal position to the vertical position, and the cooling hose was positioned into the top shaker.

All electronics were then turned on, and signal presentation commenced. During the signal presentation time, an observer was always in the room in case of equipment failure. The observer also watched the digital image of the fish to ensure that they were still alive and swimming around when they appeared in the 45° angle of view.

Control fish were put through the same process as treatment fish but without the pile driving sound. The treatment fish were exposed to one of eleven pile driving treatments (see Results). The treatments varied in SEL_{cum}, SEL_{ss}, and number of strikes, which affected the duration of the exposure. For example, for fish exposed to 1920 strikes, the exposure duration was 48 minutes, for 960 strikes it was 24 minutes.

When the exposure was completed, the equipment was turned off, the HICI-FT turned back into the vertical position, the shoulder bolts loosened, and the top shaker lifted with the winch. The fish were carefully removed with a net and placed into the transportation buckets for transfer to the barotrauma injury assessment. The water temperature inside was taken at the completion of each pile driving exposure.

All of the water from the exposure was drained out of the HICI-FT and the interior wiped with a sponge. If another exposure was scheduled, the HICI-FT tube was refilled with new conditioned water. Following the final exposure of a day, the water conditioning assembly was refilled with water from the building to prepare treated water for the next day.

Reference

MacGillivray, A., and R. Racca. 2005. *Sound Pressure and Particle Velocity Measurements from Marine Pile Driving at Eagle Harbor Maintenance Facility, Bainbridge Island, WA.* JASCO Research Ltd., Victoria, British Columbia, Canada.

APPENDIX H

HEARING STUDY METHODS AND RESULTS

Hearing Measurements

Introduction

The goals of this study were to assess the effects of exposure to high-intensity pile driving sounds on Chinook salmon physiology and to develop an understanding of the sound exposure(s) that is (are) sufficient to result in the onset of physiological impact on fish. An additional goal was to provide quantitative data that could be used by scientists, regulators, and industry to define criterion levels for tissue damage onset for use in design of pile driving projects and in making recommendations for protection of animals.

One additional goal of this project was to measure effects of exposure to sounds on hearing capabilities, however, due to technical difficulties, it was agreed by the investigators, the NCHRP advisors, and outside experts, that hearing measures would not be continued or included in this report.

The premise was to explore potential temporary hearing loss as a result of exposure to loud sounds (e.g., Popper et al., 2007). Hearing loss as a result of sound exposure is well-known in mammals and is the basis for federal regulation of sound exposure in the workplace. Temporary threshold shift (TTS) is a recoverable hearing loss that may last minutes, hours, or days after noise exposure. TTS has been demonstrated in a wide range of fish species including Chinook salmon (*Oncorhynchus tshawytscha*) (Halvorsen et al., 2009) and rainbow trout (*Oncorhynchus mykiss*) (Popper et al., 2007). It has been hypothesized that permanent threshold shifts cannot occur in fish because fishes replace damaged sensory cells (Lombarte et al., 1993; Smith et al., 2006), something that does not occur in mammals.

Auditory Evoked Potential

The auditory evoked potential (AEP) is a measure of the synchronized electrical responses in the brain that originates from the ear and central auditory regions in response to sound. The technique has been widely used for many fishes and terrestrial animals, including in human newborns. A complete description of the theory and operation of the AEP can be found in many recent papers (e.g., Casper and Mann, 2006; Popper et al., 2007; Halvorsen et al., 2009).

Several points about the AEP are critical to note. First, AEP is used for hearing assessment because it is a rapid and reliable method that does not require training of fish. In many other behavioral studies to determine hearing capabilities, fish must be trained to respond to a sound, and this takes considerable time (e.g., Tavolga and Wodinsky, 1963; Fay and Popper, 1975; Sand et al. 1976; Coombs et al., 1981). Thus, behavioral tests were not used to test noise exposure because it may take weeks to train a fish, and data are needed in exposure studies immediately after exposure. Although it would be possible to train a fish prior to exposure in order to do post-exposure hearing tests (as was done by Popper and Clarke, 1976 on goldfish), the training is time-intensive, and one might not prefer to sacrifice a fish for barotrauma after the extensive training.

Second, the AEP does not provide information about how brain processes the sound nor does it indicate if a fish would behaviorally respond to the sound. Third, whereas behavioral testing expresses the full conceptual processing by the fish and provides the lowest sound level detectable by the animal (i.e., the absolute threshold), the AEP does not indicate the lowest sound level detectable.

The AEP enables rapid assessment as to whether a fish detects a sound. Thus, the AEP can be used to compare sound detection capabilities in two fish and then determine if there are relative differences in sound detection between specimens. And, in the case of the pile driving studies, the AEP can be used to

compare sound detection between fish exposed to pile driving sounds and fish not exposed. The differences in detection would indicate if there is TTS resulting from sound exposure. At the same time, it is critical to note that the comparisons between fish, such as those done here, must be in the identical setups and with identical sounds. Otherwise, differences in acoustics in other tanks might be the cause of any variations found in hearing using the AEP method.

General Procedures for Measuring Auditory Evoked Potential

Following noise exposure (or control treatment), fish were fitted with electrodes to record the AEP. They were then placed in the hearing assessment tube (HAT) (next section) and presented with sounds of various intensities. The responses from the electrodes were amplified and carried to an A/D converter and recorded. The lowest sound that produced a recognizable response was considered the threshold or lowest sound level detectable by the animal at a particular frequency. This approach was followed for each frequency tested from 48 to 500 Hz, the hearing range of juvenile Chinook salmon determined in earlier physiological studies (Halvorsen et al., 2009).

Hearing Assessment Tube and Control

The HAT was a 1.9 cm-thick steel tube 1 m deep with an inner diameter of 31.8 m (Figure H-1). The tube was mounted onto a support frame with bolts and floated with vibration isolation springs. A U.S. Navy J-11 transducer was bolted to the bottom of the tube, facing upward into tube. The fish was placed into the tube and exposed to sounds. The sounds were controlled via a PC fitted with an A/D board through which tone signals were presented and which also recorded the AEP signals from the electrodes. The entire HAT system was controlled by the evoked response study tool (EVREST), "…a Windows-based hardware/software system designed for calibrating sound stimuli and recording and analyzing transient and steady-state evoked potentials" (Finneran, 2009, p. 491). The EVREST system has been used successfully with marine mammals (Finneran, 2009, 2010) and fish (Hastings et al., 2008). The software presented the signals and analyzed the incoming AEP signal, using an algorithm to determine threshold.

Equipment and Software Setup

The J-11 transducer was driven by an amplifier powered by a deep-cycle marine battery that was charged overnight to ensure power was maintained for an entire experiment. The battery was used to reduce electrical noise that could have interfered with the ability to detect the electrical signals coming from the fish ear and brain during AEP measurement. The system was controlled using EVREST software on a Dell laptop computer with an A/D board, a programmable attenuator, and an anti-aliasing filter. EVREST used National Instruments hardware and LabVIEW-based software. The most basic functions of the graphical user interface generate the signal and collect and store the AEP responses. The thresholds were based on an average minimum of 512 stimulus presentations, corresponding to the lowest detectable evoked response and the next-lowest undetected response. The response detection method was set for two times the center frequency using a single-point F-test, a time domain technique used for statistical response detection.

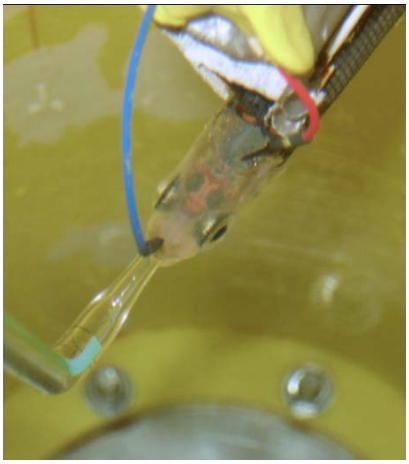


Figure H-1. Left: Hearing assessment tube (HAT). Right: Close up view of fish detailing placement of reference and recording electrodes. A tube delivers chilled, oxygenated water into the fish's mouth and over its gills.

Preparing the Hearing Assessment Tube and Fish for Experiment

The HAT water was cooled and maintained at 14°C so that the water temperature was the same as the fish had experienced in the HICI-FT tube. A fish from a HICI-FT exposure was brought into the sound chamber in a small bucket of 14°C ±0.5°C water. The fish was carefully picked up and held in a wet cloth, and injected with 10 µL of Flaxedil (20 mg/mL concentration), a neuromuscular blocking agent, into the dorsal musculature just below the dorsal fin. This agent 'froze' the body muscles to keep the fish still during the AEP tests. The fish was then carefully held in a mesh sling, with the edge of the mesh kept caudal of the operculum so that opercular movement (i.e., respiration) would be unimpeded. The top edge of the mesh was attached to a clip, which was itself attached to a PVC assembly clamped above the HAT, thereby suspending the fish.

Two electrodes were inserted carefully just under the surface of the skin. A third electrode was hung loose in the water of the HAT and served as the ground. The reference and recording electrodes were insulated with nail polish, except for the 2-mm tip. All electrodes were inserted under the skin only as far as the exposed tip ($\leq$ 2 mm). The recording electrode was inserted near the top of the head, just caudal to the skull. The reference electrode was placed between into bone by the nares. Electrodes were connected to the preamplifier and the signal carried to the PC.

The PVC pipe was then lowered so that the top of the fish's head was 19.5 cm below the water surface. The outflow tube from the upper water holding tank was maneuvered into the fish's mouth to force oxygenated, 14°C water over the fish's gills for respiration, as the Flaxedil injection prevented fish from moving their opercula on their own.

Auditory Evoked Potential Recordings

EVREST software played out stimulation tones to the fish. The computer presented a sound; if the EVREST software determined that the fish detected the sound, the SPL of the next signal was 6 dB lower. When a signal was not detected by the fish, the program increased the SPL in 1-dB increments until the fish again detected the sound. A threshold was determined when the fish was able to detect a level twice in a row.

After thresholds were determined at all test frequencies, the fish was raised to the top of the tank. The electrodes were carefully removed and the length and weight measurements of the fish were recorded on the data sheets. An example of the data sheet is shown in Appendix C.

Initially, baseline data was collected in the form of audiograms from naïve fish that came directly from holding tanks. These animals were not exposed to pile driving or used in the HICI-FT. Baseline data is used to assist with the determination of thresholds and to elucidate any effects of handling on controls when compared to the control population.

Auditory Evoked Potential Results

Figures H-2 through H-6 show comparative AEP results between treatment and control fish. Figure H-2 compares Treatment 1 control fish with the baseline group. Baseline fish were taken directly from the tank to help determine handling effects. It can be seen from the error bars in Figure H-2 that there was variation—10 dB and greater—in the results for these animals.

There is overlap between the averaged data points, but due to the large variation, it is not possible to determine whether the results reflect normal variation in fish hearing sensitivity or actual differences resulting from handling or sound exposure.

The results from the hearing studies were equivocal, with wide variation in the results for experimental (n = 135), control (n = 60), and baseline (n = 39) animals. This variation spanned as much as 38 dB, making it impossible to determine if there were effects from pile driving sound exposure. Indeed, the extent of variation in the results for control animals was as great as for experimental animals. After discussion with our external panel of fish hearing experts and with our NCHRP advisory group, it was decided that the hearing tests were not providing useable data and that it was not possible to determine the basis for the variation without an additional separate, and very extensive, set of experiments. Thus, it was agreed that the hearing studies would terminate, and that the data *could not* be used for any useful purpose in evaluating effects of sound on fish.

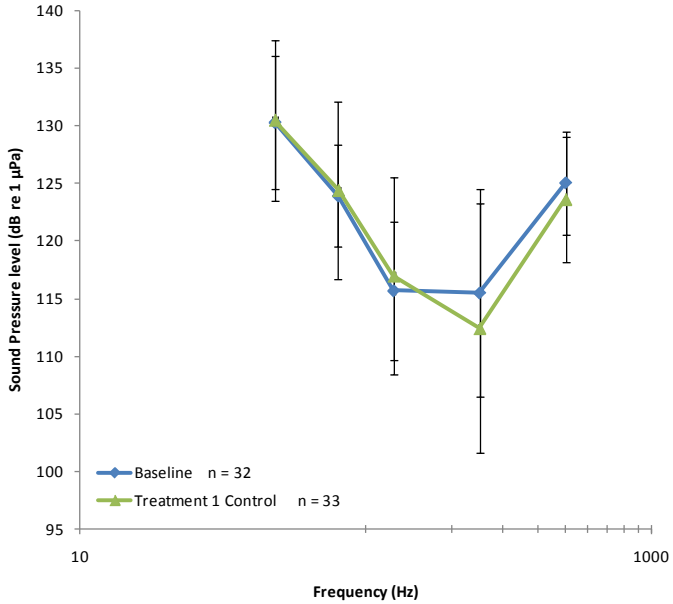


Figure H-2. Baseline compared to Treatment 1-control.

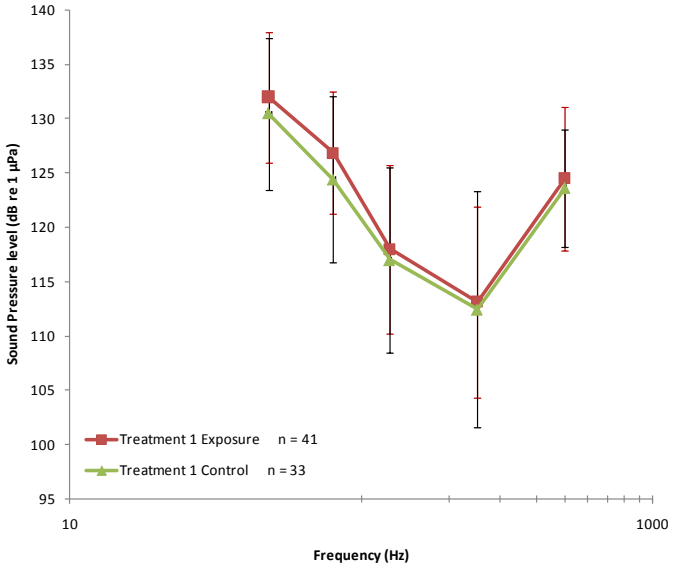


Figure H-3. Data from Treatment 1 (219 SEL_{cum} with 1920 pile strikes).

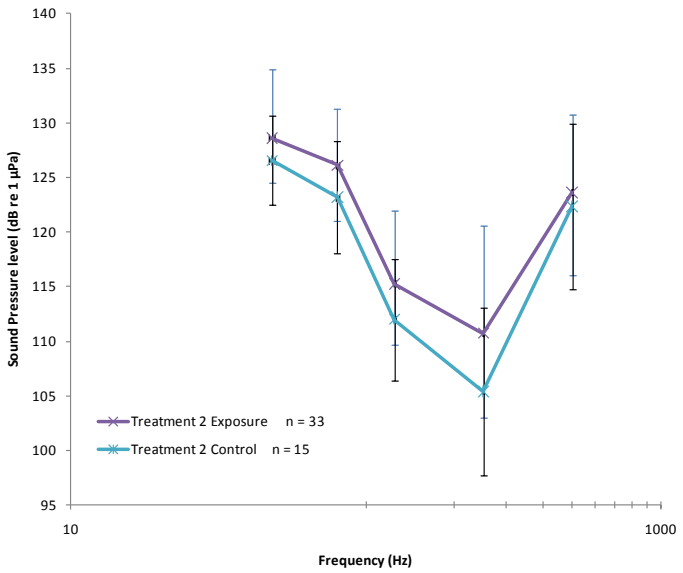


Figure H-4. Data for exposed and control animals from Treatment 2 (216 SEL_{cum} with 1920 pile strikes). The large standard deviations for both treatment and control make it impossible to determine whether thresholds are the same or different.

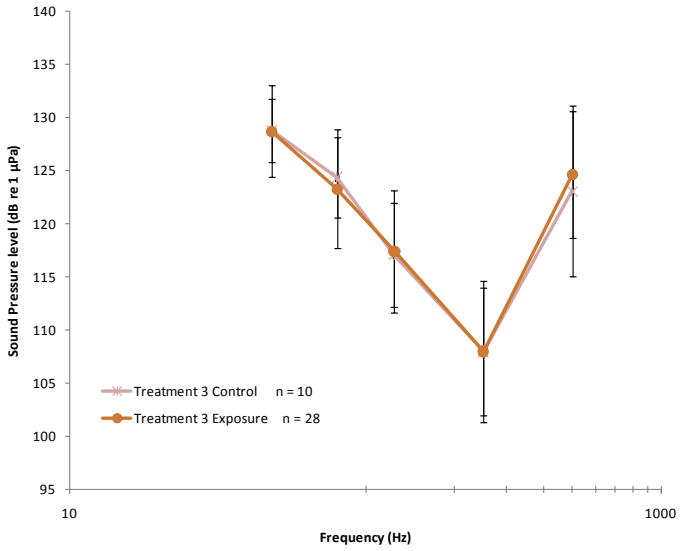


Figure H-5. Data from Treatment 3 (216 SEL_{cum} with 960 pile strikes). With the large variability at each frequency, it is not possible to make a determination of similarities or differences in hearing sensitivity resulting from sound exposure.

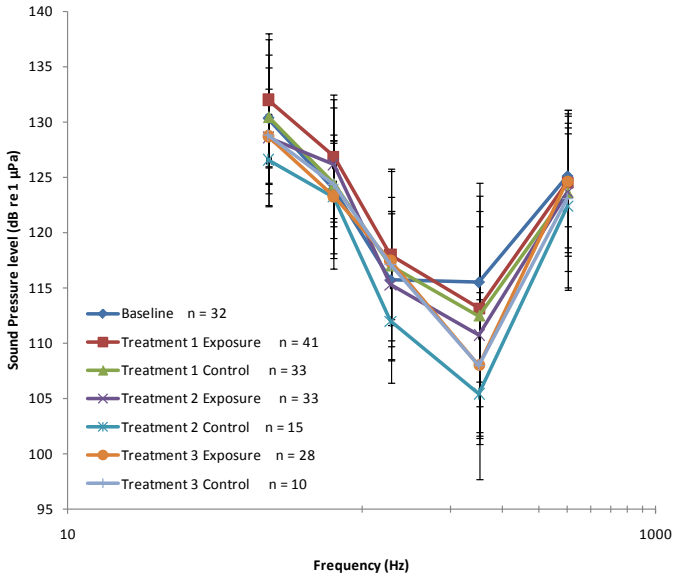


Figure H-6. Data for all control and treatment fish showing the very substantial overlap and variation with the error bars. The salient feature is the broad standard deviations at each frequency, which makes the data uninterpretable.

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