



Mad River Bridges Replacement Project Effects of Pile Driving Sound on Juvenile Steelhead

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List of Abbreviated Terms

μPa	micropascal
Caltrans CISS	California Department of Transportation cast-in-steel-shell
dB	decibels
ng/mL NMFS	nanograms per milliliter National Marine Fisheries Service
project	Mad River Bridges Replacement Project
RMS	root mean square
SEL	sound exposure level
SLM	sound level meter
SPL	sound pressure level
US 101	U.S. Highway 101

Mad River Bridges Replacement Project Effects of Pile Driving Sound on Juvenile Steelhead

Abstract

The biological effect of underwater sounds generated from anthropogenic sources on aquatic organisms has been identified as a growing concern. One sound source at issue is the underwater sound generated by pile driving in and near fish-bearing waters. Laboratory data for a variety of sound sources have been used to estimate the thresholds of effects of pile driving on fish. However, there have been few experiments that evaluate pile driving sound propagation and attendant physical effects of pile driving sound on fish in natural environments, particularly in riverine systems.

This study used caged fish deployments within the Mad River (California) to expose juvenile steelhead (*Onchorhynchus mykiss*) to a variety of peak sound pressures levels (SPLs) and cumulative sound exposure levels (SELs) from 2.2-meter-diameter (7.2-foot-diameter) cast-in-steel-shell (CISS) piles driven immediately adjacent to the Mad River. Four experimental trials were conducted. Each trial consisted of the driving of one pile section (20 to 24 meters [60 to 80 feet]). During each trial, cages containing fish were placed at four exposure locations at different distances from the pile driving activity (approximately 35 to 150 meters [115 to 490 feet] away) and at a control location (350 meters [1,150 feet] away). Underwater sound (peak and SEL) was monitored and recorded at each location during the experiments. Following cessation of pile driving, blood samples were drawn from each fish for hematocrit (i.e., packed cell volume) and plasma cortisol level, and a necropsy was performed on each fish. Organ samples were also collected for histopathology by a highly experienced fish veterinary pathologist.

During pile driving, fish were exposed to underwater peak SPLs ranging from 69 to 188 decibels (dB) relative to 1 micropascal (re 1 μPa), which is considerably lower than the interim criteria of 206 dB for peak SPL. Cumulative SELs, which in this case is a measure of the cumulative energy to which a fish is exposed to over the course of a pile driving event (total pile driving in one day), ranged from 179 to 194 dB re 1 $\mu\text{Pa}^2\text{-sec}$. The cumulative SEL exceeded the interim cumulative SEL threshold of 187 dB during the last two pile driving events, both times in the two cages closest to the pile being driven (thus, four exposure groups experienced cumulative SELs in excess of 187 dB). Control fish experienced SELs of 132 to 141 dB, far below the 150 dB threshold above which SELs are presumed to accumulate. On-site necropsies of all exposed and control fish conducted following each trial, as well as histopathology of the fish from the cages closest to the pile driving and control fish, showed no physical trauma that could be related to exposure to underwater noise from pile driving, and no statistically significant differences between experimental and control animals were detected. Similarly, hematocrit and plasma cortisol levels were not significantly related to exposure to noise generated by pile driving. In summary, there were no immediate significant physical effects of exposure to peak SPLs or cumulative SELs of ≤ 194 dB from pile driving at the project site.

Introduction

In recent years, intensive research interest has developed concerning the effects of intense, impulsive underwater sound on fishes. This research interest developed from observations of fish mortality and sub-lethal injury associated with acoustic disturbances, including seismic airguns, towed sonar arrays, pile driving, and other common sources of high-intensity underwater sound. Studies of the effects of pile driving have been particularly extensive along the northwest coast of North America because of the number of pile driving activities in waters occupied by various species of Pacific salmon that are protected under the federal Endangered Species Act as threatened and endangered. Studies to date have, however, been hampered by a lack of uniformity in methodology, lack of appropriate controls, poor analysis of tissue post exposure, and, in many cases, by an experimental design that fails to consider sources of variability that heavily influence fish response to underwater sound (Popper and Hastings 2009). This study endeavored to precisely quantify both fish exposure to underwater sound and a variety of potential physiological effects of that exposure, as well as ensure that many of the concerns raised about earlier field studies of the same basic type did not arise during this study.

The primary objective of this study was to test whether juvenile salmonids would be harmed by exposure to sound generated by pile driving. Specifically, this included testing of the following alternative hypotheses:

- **H0:** Death or injury of fish exposed to accumulated underwater sound generated from pile driving during the experiment is the same as death or injury occurring in control fish.
- **H1:** Death or injury of fish exposed to accumulated underwater sound generated from pile driving during the experiment is greater than death or injury occurring in control fish.

The study was performed in the context of the Mad River Bridges Replacement Project (project), a California Department of Transportation (Caltrans) project to replace the U.S. Highway 101 (US 101) bridges crossing the Mad River north of Arcata, California (Figure 1). This study focused on the driving of two of the three 49-meter-long (160-foot-long), 2.2-meter-diameter (7.2-foot-diameter) CISS piles at Pier 3 during July and August 2009. The piles were driven with a Pileco D225 diesel impact hammer, at the time the largest impact hammer used in the United States. Each pile was driven in two sequential drives. The first pile section was driven approximately 20 meters (65 feet) into the ground. After welding the second section to the first, the completed pile was driven an additional 24 meters (80 feet) into the ground. Different groups of juvenile steelhead (*Oncorhynchus mykiss*) were exposed during each of four different pile drives (two first-section and two second-section drives) within cages placed in the river at distances from 35 to 150 meters (115 to 490 feet) from the driven piles, and also at a control cage located 350 meters (1,150 feet) from the driven piles. Although the control cage was exposed to some underwater sound, the presence of an intervening shallow water depths and the meander of the river retarded inwater transmittance of underwater sound; sound levels at the control cage were monitored during the experiment to verify this fact. No more distant location was available for a control cage because of a long reach of shallow water upstream of the 350-meter (1,150-foot) pool and the total absence of a suitable downstream location. The peak and cumulative underwater sound levels were measured at each cage, and following the exposure to underwater

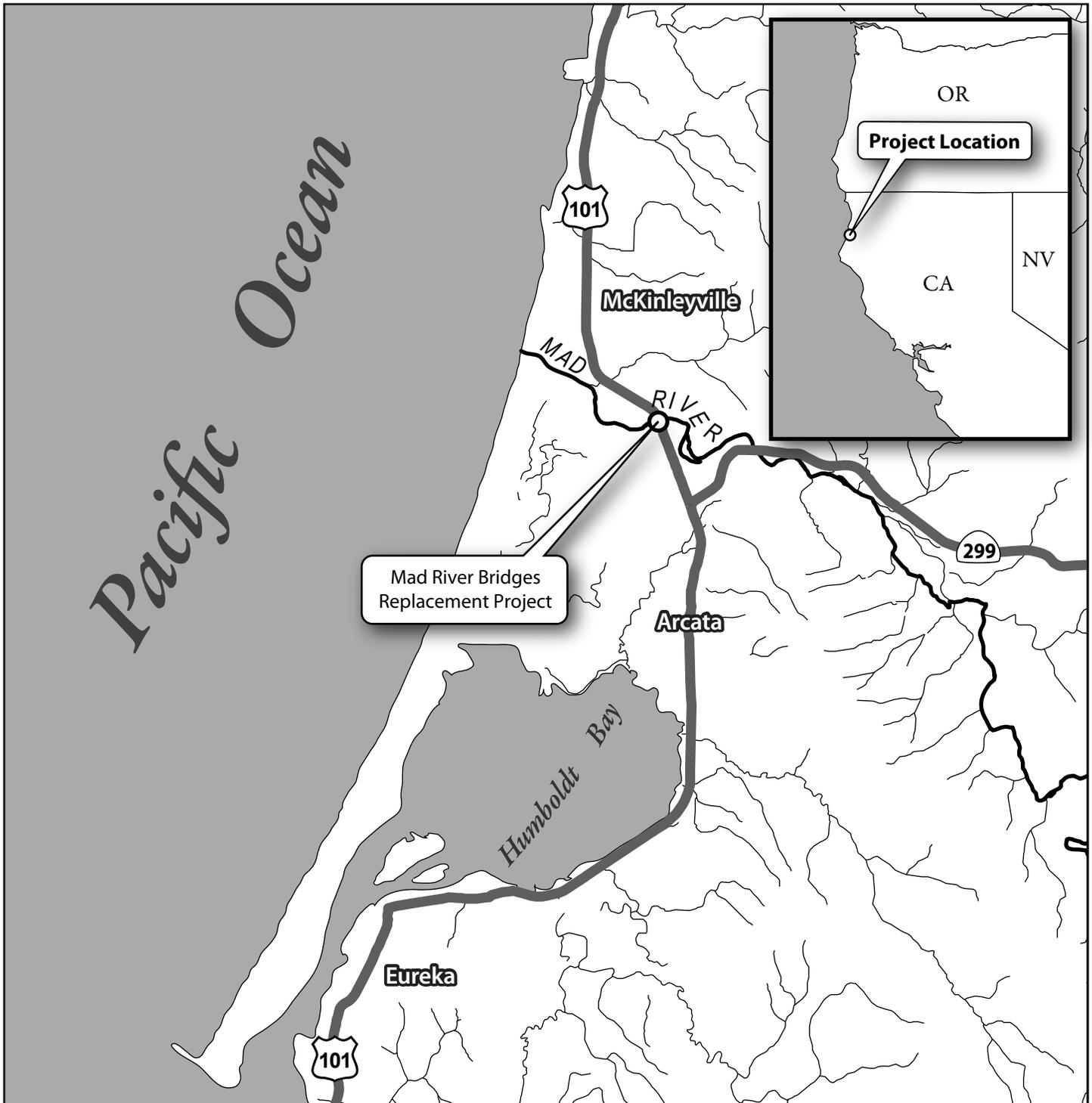


Figure 1. Project Location and Vicinity

Mad River Bridges Replacement Project

pile driving sound, the fish were necropsied to look for physical injury, and organs from a subset of the fish underwent subsequent histopathology.

Study Area

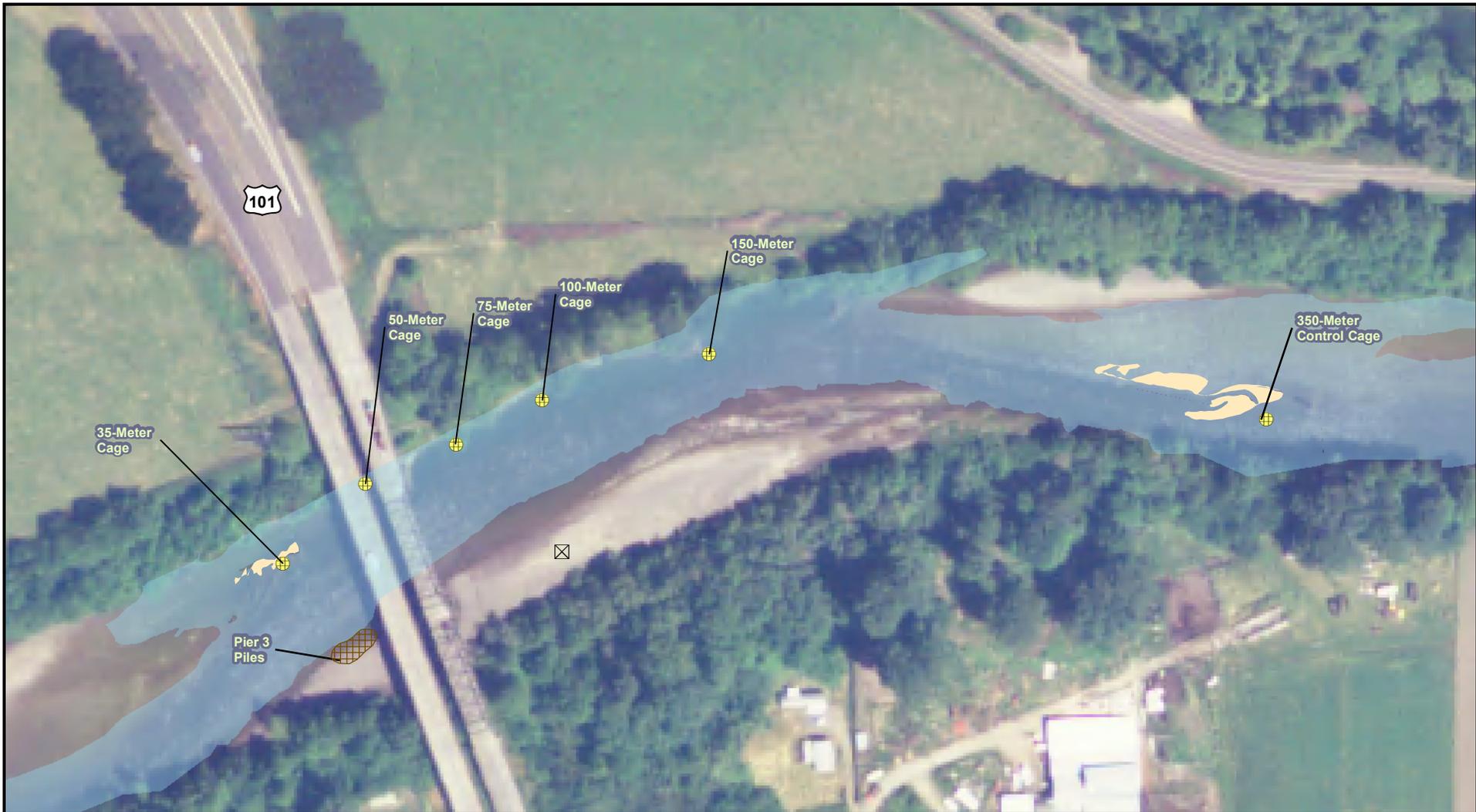
The project is located on the Mad River where US 101 crosses the river between Arcata and McKinleyville, California (Figure 1). The location of piles driven during the experiment was immediately south of the existing bridges. The fish cage deployments occurred adjacent to and upstream of the piles, at stations named according to their distance in meters from the pile driving: 35 meters, 50 meters, 75 meters, 100 meters, and 150 meters (115, 164, 246, 328, and 492 feet) from the piles; the control cage was at the 350-meter (1,150-foot) station upstream of the piles (Figure 2).

Methods

The objective of the study was to expose fish to underwater sound generated by pile driving and simultaneously measure the peak and accumulated underwater SPLs received by the fish. Following exposure, necropsies and histopathology of the fish were conducted to determine whether exposure resulted in physical injury to the fish.

During summer, the Mad River at the project site is relatively shallow, with broad gravel runs that typically measure 0.6 meter (2 feet) deep or less, except along the north bank, where a 1.0- to 1.3-meter-deep (3- to 4-foot-deep) channel extends from just east of the bridges to approximately 180 meters (600 feet) upstream. During the first pile driving trial, four cages containing fish and their respective hydrophones were set in this channel at the 50-meter, 75-meter, 100-meter, and 150-meter stations (Figure 2). The control station was located in a small pool approximately 350 meters (1,150 feet) upstream of the piles. Each station consisted of a frame set into the streambed, to which were attached a cage containing the fish and an identical paired cage containing the hydrophone. After the first pile driving trial, a small 1.0-meter-deep (3.3-foot-deep) pool was located approximately 35 meters (115 feet) from the piles. This location had not been selected initially because of the shallow water depth; all other cages were located in water deep enough for the center of the cage to be at least 1.1 meter (3.6 feet) from the bottom and for the cage to remain submersed during the 0.2-meter (0.6-foot) daily tidal fluctuations (thus, a water depth of 1.3 meters [4.2 feet]). However, observed absence of any superficial behavioral change or trauma in fish at the 50-meter cage during the first trial prompted a decision to try to site a cage even closer to the pile driver. Accordingly, a cage and hydrophone were relocated from the 100-meter station to the 35-meter station. Therefore, for the second, third, and fourth pile driving trials, the exposure cages were located at the 35-meter, 50-meter, 75-meter, and 150-meter stations, with the control cage still at 350 meters.

The cages used for the experiment were 0.8-meter-long by 0.23-meter-diameter (31-inch-long by 9-inch-diameter) vinyl-coated Frabill Model 1273 crawfish trap, modified by sealing each end to contain fish. A small plastic bucket was attached to the upstream end of each cage to provide a flow refuge for fish. Identical cages were constructed to house the hydrophones. On each cage



Source: CalTrans 2005 (Aerial Photograph); ICF Jones & Stokes (Wetted Extent Mapping, September 3, 2009)

Legend

-  Island
-  Wetted Extent, September 2009
-  Cage
-  Project Pathology Lab



Figure 2. Cage Locations

Mad River Bridges Replacement Project
Arcata, CA

stand, a cage containing fish and a cage containing a hydrophone were mounted immediately next to one another (Figure 3).

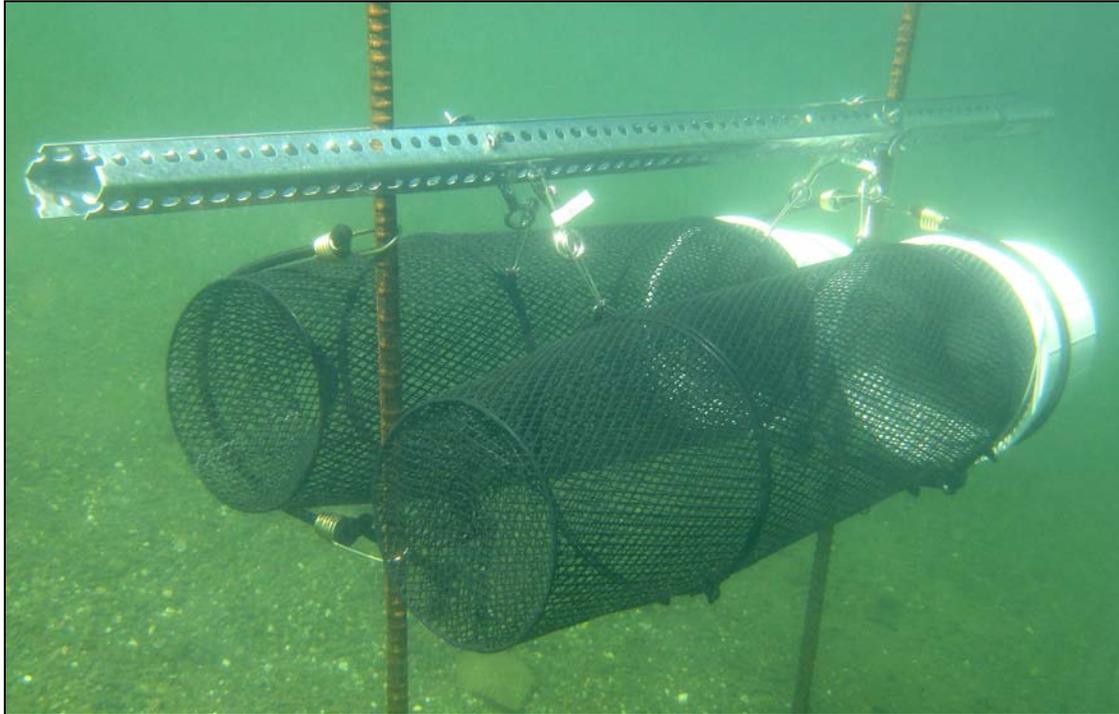


Figure 3. Fish and Hydrophone Cages on a Cage Stand

Hydroacoustic Monitoring

Hydroacoustic Terms

Several hydroacoustic terms are used in this report:

- **Peak SPL:** The maximum absolute value of the instantaneous sound pressure that occurs during a specified time interval, measured re 1 μPa .
- **Root mean square (RMS) SPL:** The square root of the mean square pressure. This term describes the squared pressures over the time that make up that portion of the waveform containing 90% of the sound energy of the impulse. This measure is used most commonly to address continuous noise sources.
- **Single-strike SEL:** The integral over time of the squared pressure of a transient waveform, in dB re 1 $\mu\text{Pa}^2\text{-sec}$. This is an approximation of the sound energy in the pulse.
- **Cumulative SEL:** Cumulative SEL is defined as the cumulative amount of energy to which a receiver is exposed over the course of a pile driving event or several pile driving events in a day. Cumulative SEL can be measured with the hydroacoustic measurement systems and can be estimated from the single-strike SEL as follows:

$$\text{SEL}_{\text{Cumulative}} = \text{SEL}_{\text{Single-strike}} + 10\log(\# \text{ of pile strikes})$$

Underwater Sound Measurement and Recording

Detailed methodology and hydroacoustic monitoring data for the project are presented in the hydroacoustic monitoring report (California Department of Transportation 2010a).

For the caged fish experiments, hydrophones were fitted into cages identical to the cages housing the experimental fish and deployed immediately adjacent to the cages containing the exposure and control fish (Figure 3). A cable connected the hydrophone to data processing and recording equipment located on the adjacent gravel bar. Each station was independent of the others; therefore, five stations were simultaneously recording during each trial, from before the initiation of pile driving until after the drive had concluded. Underwater sound was measured using G.R.A.S. Model CT10 or Reson Model 10CD hydrophones with PCB Model 422E13 in-line charge amplifiers and PCB Model 480M122 multi-gain signal conditioners. The signals were fed into Larson Davis Model 820 integrating sound level meters (SLMs) and Marantz Model PMD660 solid-state recorders. These instruments allowed precise measurement of unweighted peak SPL, single-strike SEL, and cumulative SEL. A Larson Davis Model 820 SLM was used to capture the RMS. The peak pressure and SELs were measured as unweighted peak sound pressure using the SLM, with SELs calculated and recorded during the pile driving event. Subsequent analysis of the acoustical pulses was performed using a Larson Davis Model 3000 real-time analyzer to capture the RMS pulse levels and verify the peak and SEL data. The real-time analyzer provided narrow-band frequency and waveform analyses.

The hydroacoustic measurement systems were calibrated before use in the field with a certified calibrated G.R.A.S. Model 42AA pistonphone and appropriate hydrophone coupler. The pistonphone coupler system produces a continuous 145.3- or 136.4-dB re 1 μ Pa tone at 250 hertz. This tone was measured and recorded at the beginning and end of each recording session.

Fish Source, Transport, and Deployment

Juvenile steelhead for the study were obtained from the California Department of Fish and Game's Mad River Hatchery in Blue Lake, California. The fish used for the study were variable in size ranging from 55 to 117 millimeters fork length and 1.49 to 17.43 grams. No two groups of fish (control or cage, during any of the four trials) were significantly different in length or weight (at $p = 0.05$, t-test for difference in means). At approximately 8 a.m. on the day before each experimental trial, a group of study fish was collected at the hatchery and moved by net and bucket to a 120-liter (35-gallon) truck-mounted isolation tank provided with an aerator and circulating pump. Also at this time, 10 fish were sacrificed for necropsy (hatchery control). The fish were then driven approximately 15 minutes to the study site. Upon arrival at the study site, a further 10 fish were sacrificed for necropsy analysis (transport control). By the conclusion of each experimental trial, all fish were necropsied, so a new group of fish was used in each trial.

The remaining fish were deployed to cages, with 10 fish in each of six cages. Five of these cages were to be placed at the five stations described above; the sixth was an overnight control cage, described below, placed at the 75-meter station. Fish were loaded into cages that were in 5-gallon plastic buckets containing hatchery water. The buckets and cages were then placed in the river for approximately 10 minutes to allow partial temperature equilibration. River water was then slowly added to the buckets, and the cages were carried in the buckets to the cage

stands, where they were deployed in the river. The sequence of deployment was randomized in each trial. Deployment was completed early in the day when river temperatures were within the range of 16°C to 18°C, which is close to the temperature of the hatchery water (14°C). The daily peak temperatures in the river at the locations of the fish cages were in the range of 20°C to 22°C. The deployed cages were in waters 1.05 to 1.40 meters (3.4 to 4.6 feet) deep, with the top of the cages approximately 10 centimeters (4 inches) below the water surface at low tide. Fish remained in the cages overnight to acclimate to river conditions. The following morning, before pile driving, fish in the overnight control cage were sacrificed for necropsy. Also at this time, fish in the other cages were observed to confirm that none were dead or exhibiting anomalous behavior. Pile driving then occurred.

Pile Driving

Pile driving was performed using the Pileco D225 diesel impact hammer. The four experimental trials were conducted during pile driving on July 1, 6, 8, and 10, 2009. The duration of the pile driving, timing of pile blows, and force of each blow varied widely between the four measured drives. Therefore, the four trials represent independent events, rather than experimental replicates.

Cage Recovery

Cage recovery began immediately following the completion of pile driving for each day. At recovery, each cage was inspected *in situ* for 1 to 2 minutes to observe fish behavior and note any irregularities (e.g., fish moribund, fish swimming irregularly, fish clustered at bottom or back of cage, fish darting around). However, no such irregularities were observed. In each case, the cage closest to the pile driving location was recovered first and the fish therein sacrificed, subjected to a field necropsy, and sampled for later histopathology. Next, the control cage at 350 meters was recovered and its fish processed, with the remaining cages subsequently recovered in a randomized sequence. It typically took approximately 40 to 50 minutes for the pathologist to complete the field necropsies, collect blood and organ samples from each cage of fish, and prepare the field laboratory for the next lot of fish. Generally, a period of approximately 6 hours elapsed from the conclusion of pile driving trials to the recovery of the final fish cage from the river.

Specimen Handling and Pathology

All pathological work was performed by Gary D. Marty, DVM, Ph.D., a pathologist highly experienced in analysis of juvenile salmonids. Detailed methodology and results of the necropsy and histopathology are presented in the pathology report (California Department of Transportation 2010b).

All exposure (n = 159) and control fish (n = 156) were individually euthanized with MS-222 (tricaine methanesulfonate) buffered in accordance with Office of Laboratory Animal Welfare (2002) standards, reviewed for any gross exterior pathology, weighed and measured, and

necropsied.¹ Gross observations and necropsy included condition of the skin, eyes, fins, mesenteric vasculature, and swim bladder, as well as general observations for parasites on the skin and gills (Table 1). Blood samples were drawn from the caudal vein to determine hematocrit and plasma cortisol levels. Histology was performed on the fish from the exposure cages closest to the pile driving locations (four cages, n = 40). In addition, two cages (n = 20) at 50 meters that received cumulative SELs in excess of 187 dB during the last two trials were examined for histopathology (total number of exposure fish undergoing histopathology = 60). All exposure control groups (n = 37²) were also examined for histopathology. Samples prepared for histological examination included head, gill, liver, and a body wedge comprising the swim bladder, trunk kidney, spinal cord, vertebrae, skeletal muscle, and skin. Tissue samples were preserved in 10% neutral-buffered formalin and shipped to the laboratory for processing using standard histological methods. Histological samples on glass slides were observed using light microscopy. Slides were processed blindly so that the attending pathologist would not know the experimental group of the sample. The pathologist ranked each tissue sample based on a scale of 0 (no histological effect on tissue being analyzed) to 3 (significant effect to the tissue).

Table 1. Pathological Conditions Evaluated

Pathological Procedure	Conditions Evaluated
Necropsy	<ul style="list-style-type: none"> • Length (millimeters) • Weight (grams) • Caudal fin fraying • Caudal fin reddening • Other fin fraying • Fin base reddening • Focal skin reddening • Visceral cavity hemorrhage • Liver hemorrhage • Swimbladder hemorrhage • Kidney hemorrhage
Blood testing	<ul style="list-style-type: none"> • Hematocrit (packed cell volume [%]) • Plasma cortisol (nanograms per milliliter)
Histopathology	<ul style="list-style-type: none"> • Gill histopathology <ul style="list-style-type: none"> – Lamellar hyperplasia/hypertrophy – Lamellar telangiectasis – Ciliate parasites – Granulomatous inflammation • Liver histopathology <ul style="list-style-type: none"> – Hepatocellular glycogen – Hemorrhage (not detected) – Lipidosis – Focal/multifocal parenchymal leukocytes (hepatitis) – Cholangitis/pericholangial leukocytes – Perivascular lymphocytes/leukocytes – Hepatocellular megalocytosis • Kidney, swimbladder, and body wedge histopathology <ul style="list-style-type: none"> – Hematopoietic cells (relative area) – Tubular epithelial protein (intracytoplasmic) – Granulomatous inflammation – Swimbladder hemorrhage – Skeletal muscle/skin hemorrhage • Brain/head histopathology <ul style="list-style-type: none"> – Hemorrhage in brain (not detected) – Hemorrhage in other head structures – Granulomatous inflammation – Lymphocytic inflammation

Analysis

Four cages were exposed on each of four pile driving trials, yielding 16 values of the independent variable. The exposure control fish population consisted of one cage per day on

¹ Three fish from the exposure control group and one fish from the exposure group escaped during the first trial. Cage closures were tightened after the first trial, and no fish escaped during the latter three trials.

² Three fish from the exposure control cage escaped during the first trial.

each of 4 days. Necropsies were also performed on the hatchery, transport and overnight control fish.

For histopathology, six exposure cages (one each for the first and second trials, and two each for the third and fourth trials) were used, yielding six values of the independent variable. The histopathology analysis used fish from the cages located closest to the pile driver, which would presumably be exposed to the highest sound levels. Analysis of the acoustic impulse data later showed that this assumption was true. The four exposure control cages (one for each trial) also underwent histopathology.

Previous studies of how caged fish respond to sound have identified a range of dependent variables, all of which constitute measures of fish response. They include fish mortality—either during the test or during an observation period after the test (Govoni et al., 2003, 2008)—and various conditions identified during necropsy and histology Abbott et al. (2005) identified 32 such conditions, and Govoni et al. (2003) identified eight such conditions. Popper et al. (2007) state that they examined the fish via various necropsy and histological conditions, with each condition scored for severity. Neither Abbott et al. (2005) nor Popper et al. (2007) found significant differences between the control and treatment groups regarding the physical conditions evaluated. However, Govoni et al. (2003) found numerous differences between control and treatment fish. They regarded the following conditions as indicating that a fish had been injured by ensonification: death, being stunned, partial evisceration, autolysis of viscera, swim bladder hyperemia, liver hyperemia, hematuria, coagulative liver necrosis, and ruptured pancreas. Based on the results of Govoni et al. (2003, 2008), a wide array of conditions were included as dependent variables in the study design. Each dependent variable was scored and summed to include all fish in each cage. It should be noted, however, that Govoni et al. (2003) used a very different stimulus than used in other studies (an explosion), so the source of damage to the fish (explosive concussion), rather than the accompanying sound, could have been the source of the damage in that study. Therefore, Govoni represents a “worst-case” scenario with a stimulus that is very different than pile driving, seismic air guns, or sonar.

For this study, the incidence of fish exhibiting a particular condition (score greater than zero) in each exposure cage was compared to that expressed by their exposure control group. Results were evaluated statistically using Fisher’s exact test for the categorical variables (detection of pathological states) and Student’s t-test for continuous variables (i.e., fish length, fish weight, hematocrit, and blood cortisol). All statistical tests used in this study were two-sided, except the Student’s t-test for cortisol, where the alternative hypothesis was one of increased cortisol. Quantile plots were used to confirm the assumption of normal distribution for the continuous variables; Fisher’s exact test assumes only that the dependent variables in the test and control cages have the same distribution, an assumption that seems well-supported for this type of investigation. Results were considered significant at $p \leq 0.05$.

Results

Pile Driving Trial Summary

Pile driving trials during which fish exposures were conducted occurred on four different days. Summary information for the four trials is presented in Table 2. The time required to perform pile driving varied from 2 hours and 6 minutes to 4 hours and 30 minutes. The first two pile sections, driven approximately 65 feet, went in relatively easily and quickly, requiring 1,100 and 1,164 pile blows. The second two drives, driven approximately 80 feet, required many more blows (4,306 and 3,396) that were individually more powerful because of increased density of the substrate (sandy to gravelly river alluvium) with increasing pile depth. Details of pile driving activity and hydroacoustic monitoring are presented in the hydroacoustic monitoring report (California Department of Transportation 2010a).

Table 2. Summary of Pile Driving Activity for Each Experimental Trial

Measurement	Drive Date (2009)			
	July 1	July 6	July 8	July 10
Duration of pile driving	09:41 to 11:47	12:12 to 15:29	11:48 to 17:19	8:33 to 11:53
Number of hammer strokes	1,100	1,164	4,306	3,396
Number of drive periods	2	2	3	4
Duration of fish recovery	12:00 to 18:44	15:42 to 22:15	17:25 to 00:10 (July 9)	12:30 to 19:38

Exposure to Underwater Sound

Underwater sound levels recorded at the test and control cages are summarized in Table 3 and illustrated in Figures 4 and 5. Assuming that, per National Marine Fisheries Service (NMFS) guidelines, sound is not accumulated for single-strike SEL values of 150 dB or less, only seven values of the independent variable (cumulative SEL) occurred. The remaining cages did not achieve single-strike SEL values of more than 150 dB. In each pile driving trial, cumulative SEL values were observed only at the 35- and 50-meter stations, which were closest to the pile being driven. Single-strike SEL levels measured at the exposure control cage (350-meter station) were very low, ranging from 132 to 141 dB SEL for the four pile driving events (Table 3 and Figure 5).

Based on the peak values (Table 3 and Figure 4), the average sound reduction from the 50-meter station to the 75-meter station varied from 12 to 20 dB, the reduction from the 50-meter station to the 150-meter station varied from 12 to 17 dB, and the reduction from the 50-meter station to the 350-meter station (the exposure control) varied from 24 to 30 dB. These attenuation rates are far in excess of the 4.5 dB per doubling of distance that would be predicted using the practical spreading model advocated by NMFS. The measurements in this study indicate that the sound energy received at the 75-meter and 150-meter stations was no more than 25% of that received at the 50-meter station, and sound energy received at the control station was no more than 6.25% of that received at the 50-meter station. Sound did not attenuate simply in proportion to distance; in some cases, peak dB and/or single-strike SEL were greater at the 50-meter station than at the 35-meter station, and in three trials, both peak dB and single-strike SEL were slightly greater at the

150-meter station than at the 75-meter station. This result may be a consequence of resonance effects or reflection/refraction effects in this setting, where most of the path length between the pile and the fish cage is through river sediments, rather than through the water column. The water depth between the piles and the stations where sound was monitored is typically very shallow (0 to 1 meter [0 to 3.3 feet]), except in the north and south bank channels, where the depth increases to approximately 3 meters (10 feet).

Table 3. Hydroacoustic Data Collected during Caged Fish Study

Date (2009)	Cage Distance from Pile (Meters)	Peak dB	Single-Strike SEL (dB)	Cumulative SEL (dB)
July 1	50	183	158	185
	75	163	139	—*
	100	165	142	—*
	150	171	148	—*
	350 (control)	153	134	—*
July 6	35	186	158	185
	50	180	154	179
	75	164	140	—*
	150	167	143	—*
	350 (control)	156	132	—*
July 8	35	185	160	194
	50	188	161	194
	75	169	146	—*
	150	171	148	—*
	350 (control)	162	141	—*
July 10	35	188	161	194
	50	181	156	188
	75	169	144	—*
	150	165	142	—*
	350 (control)	154	133	—*

* Single-strike SELs less than 150 dB do not accumulate to cause injury to fish, per National Marine Fisheries Service guidelines.

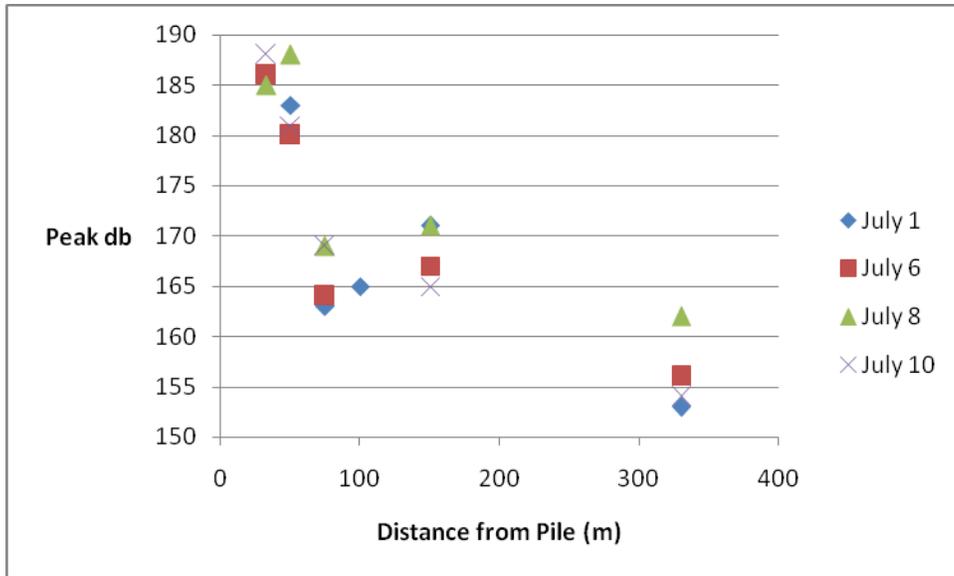


Figure 4. Comparison of Peak Sound Levels Recorded at Caged Fish Locations at Mad River in 2009

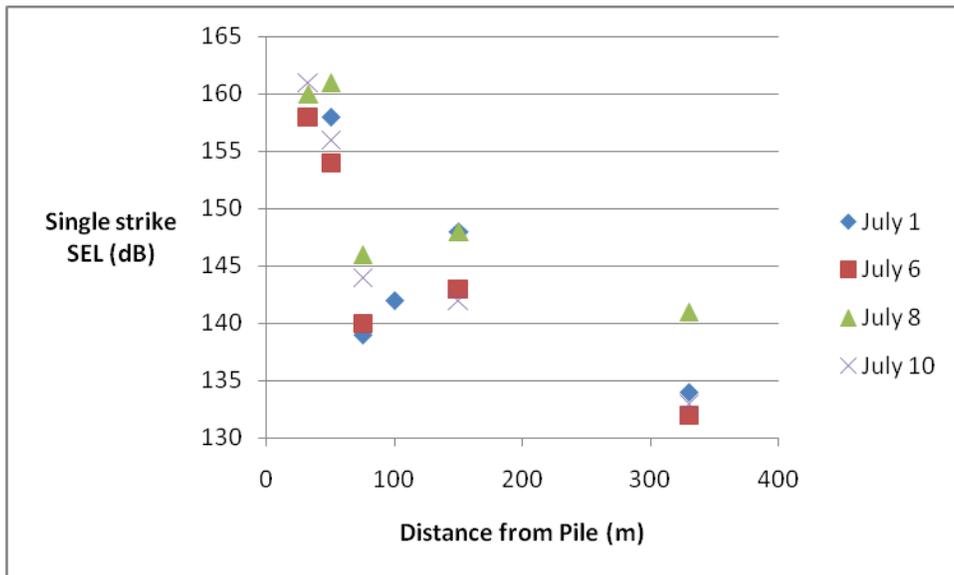


Figure 5. Comparison of Single-Strike SELs Recorded at Caged Fish Locations at Mad River in 2009

Condition of Exposed Fish

Survival

No fish died in any of the exposure or control cages. Further, no fish in exposure or control cages exhibited distress or abnormal behaviors when observed in the cages after pile driving trials.

Internal and External Injury

There were no statistically significant differences between the incidence of conditions in exposed and control fish in the conditions evaluated during necropsy or histopathology. This is true of the exposure controls as well as the hatchery, transport, and overnight controls, although only the exposure controls were subject to histopathological analysis. Hematocrit and plasma cortisol levels are used as an indication of stress in fish. Although there were statistically significant differences between some of the exposed and control fish groups for plasma cortisol and hematocrit levels, these differences appeared to be more likely associated with fish handling and confinement than exposure to pile driving noise (see below).

The analysis looked for but did not detect any of the following conditions in fish that underwent necropsy: caudal fin reddening, focal skin reddening, visceral cavity hemorrhage, liver hemorrhage, swim bladder hemorrhage, and kidney hemorrhage.

Fin fraying (caudal fin and other fin), which is fairly common in hatchery fish, was fairly common and minor to moderate in the exposure and control groups. The incidence and severity of conditions observed during necropsy are presented in the pathology report (California Department of Transportation 2010b).

Relatively few other conditions were detected in either the necropsied fish or the fish that underwent more detailed histopathology (see Table 4 for summary data and the pathology report [California Department of Transportation 2010b] for a detailed reporting of findings). The conditions detected (and not detected) in the histopathology evaluation are listed in Table 4. As noted in Table 4, only 20 of a possible 35 types of conditions were detected, with 124 noted in the 37 control fish (an average of 3.4 per fish) and 198 noted in the 60 exposed fish (an average of 3.3 per fish). None of the conditions showed a statistically significant difference between fish from the exposed and control cages. Apart from fin fraying, as noted above, the most prevalent (but not statistically different) states were enlarged liver cells (hepatocellular megalocytosis), foci of inflammation in the liver (focal/multifocal parenchymal leukocytes), inflammation around the ducts that carry bile from the liver to the gallbladder (cholangitis/pericholangial leukocytes), and granulomatous inflammation in several organs. These conditions are consistent with low levels of lesions common in any fish population and with the history of a filamentous bacterial infection at the hatchery about 1 month before the project began.

Two other conditions—hepatocellular glycogen and relative area of hematopoietic cells—were noted in nearly all exposed and exposure control fish. The mean score for hepatocellular glycogen was slightly greater (1.58 versus 1.43) for exposure than for exposure control fish, but comparisons within each trial were not consistent. The observed hepatocellular glycogen condition could result from a lack of feeding for 24 hours, but no baseline is available because hatchery control fish were not analyzed for hepatocellular glycogen. The cause of hematopoiesis is unclear.

Table 4. Observed Condition of Juvenile Hatchery Steelhead Exposed to Pile Driving Noise and Its Statistical Significance (Histopathology Group)

Condition Evaluated	Description or Incidence of Condition in Fish										Test Statistics (p values)					
	Trial 1		Trial 2		Trial 3			Trial 4			Trial 1	Trial 2	Trial 3		Trial 4	
	Station 1	Control	Station 0	Control	Station 0	Station 1	Control	Station 0	Station 1	Control	Station 1	Station 0	Station 0	Station 1	Station 0	Station 1
Cumulative SEL Exposure (dB)	185	**	185	**	194	194	**	194	188	**	185	185	194	194	194	188
Description of fish																
Number of fish	10	7	10	10	10	10	10	10	10	10	–	–	–	–	–	–
Length: mean (mm)	75	80	73	78	77	79	77	78	74	74	0.38	0.48	0.94	0.63	0.27	0.94
Length: standard deviation	12	11	9	16	9	9	13	8	9	9	–	–	–	–	–	–
Weight: mean (g)	4.5	5.5	4.1	5.4	4.7	5.6	5.1	5.1	4.4	4.2	0.42	0.39	0.72	0.71	0.24	0.71
Weight: standard deviation	2.6	2.2	1.5	4.4	1.6	1.9	3.1	1.6	1.7	1.6	–	–	–	–	–	–
Blood^a																
Hematocrit (packed cell volume [%])	41	38	42	41	42	40	43	40	41	41	0.37	0.58	0.43	0.05	0.54	0.82
Plasma cortisol (ng/mL)	76	39	62	64	68	70	37	77	58	64	0.05	0.45	0.04	0.03	0.24	0.37
Gross necropsy																
Caudal fin fraying	3	2	3	5	4	4	5	4	2	4	1.00	0.65	1.00	1.00	1.00	0.63
Other fin fraying	0	0	0	1	2	2	3	3	4	2	1.00	1.00	1.00	1.00	1.00	0.63
Gill histopathology^b																
Lamellar hyperplasia/hypertrophy	0	0	0	0	1	1	0	0	0	1	1.00	1.00	1.00	1.00	1.00	1.00
Lamellar telangiectasis	0	1	0	1	0	1	0	0	1	0	0.41	1.00	1.00	1.00	1.00	1.00
Ciliate parasites	0	0	0	0	0	0	0	1	0	0	1.00	1.00	1.00	1.00	1.00	1.00
Granulomatous inflammation	0	0	1	0	0	0	0	1	0	0	1.00	1.00	1.00	1.00	1.00	1.00
Liver histopathology																
Hepatocellular glycogen	8	5	8	10	10	10	9	10	9	10	1.00	0.47	1.00	1.00	1.00	1.00
Hemorrhage (not detected)	0	0	0	0	0	0	0	0	0	0	–	–	–	–	–	–
Lipidosis	0	0	1	1	0	0	1	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
Focal/multifocal parenchymal leukocytes (hepatitis)	1	1	0	1	2	0	0	1	0	1	1.00	1.00	0.47	1.00	1.00	1.00
Cholangitis/pericholangial leukocytes	2	0	1	1	1	3	3	1	3	1	0.49	1.00	0.58	1.00	1.00	0.58
Perivascular lymphocytes/leukocytes	0	0	0	0	0	0	0	0	0	1	1.00	1.00	1.00	1.00	1.00	1.00
Hepatocellular megalocytosis	0	0	1	1	1	0	1	0	1	1	1.00	1.00	1.00	1.00	1.00	1.00
Kidney, swimbladder, and body wedge histopathology^c																
Hematopoietic cells (relative area)	10	7	9	10	10	10	10	10	10	10	1.00	1.00	1.00	1.00	1.00	1.00
Tubular epithelial protein (intracytoplasmic)	0	1	4	2	2	3	1	3	3	2	0.41	0.63	1.00	0.58	1.00	1.00
Granulomatous inflammation	0	0	2	1	0	0	0	2	0	0	1.00	1.00	1.00	1.00	0.47	1.00
Swimbladder hemorrhage	1	0	2	1	0	0	2	0	0	0	1.00	1.00	0.47	0.47	1.00	1.00
Skeletal muscle/skin hemorrhage	0	0	0	1	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	1.00
Brain/head histopathology																
Hemorrhage in brain (not detected)	0	0	0	0	0	0	0	0	0	0	–	–	–	–	–	–
Hemorrhage in other head structures	0	1	0	0	0	0	0	0	0	0	0.41	1.00	1.00	1.00	1.00	1.00
Granulomatous inflammation	0	1	3	0	0	0	0	0	0	0	0.41	0.21	1.00	1.00	1.00	1.00
Lymphocytic inflammation	1	0	0	0	0	1	0	0	0	1	1.00	1.00	1.00	1.00	1.00	1.00
Total incidence of conditions scoring <0	26	19	35	36	33	35	35	36	33	34	–	–	–	–	–	–

Notes:

Incidence = number of fish with condition score greater than zero

^a Conditions surveyed for and not detected in any fish included caudal fin reddening, fin base reddening, focal skin reddening, visceral cavity hemorrhage, liver hemorrhage, swimbladder hemorrhage, and kidney hemorrhage.

^b Conditions surveyed for and not detected in any fish included lamellar fusion and lamellar subepithelial edema.

^c Conditions surveyed for and not detected in any fish included hemorrhage, congestion, tubular dilation of lumen, and spinal cord hemorrhage.

** Single Strike SELs below 150dB

No statistically significant differences were noted in the continuous variables (Table 4), except in the blood variables, hematocrit and plasma cortisol. Plasma cortisol was elevated ($p = 0.03$ to 0.05) in the first trial and at the 35-meter and 50-meter stations in the third trial, but was not significantly elevated in the second and fourth trials. In the first and third trials, plasma cortisol was quite low (39 and 37 nanograms per milliliter [ng/mL], respectively) at the control cages, whereas in all other cases it was relatively high (58 to 77 ng/mL in test cages, and 64 ng/mL at both the second and fourth trial controls). The first group of fish tested at the hatchery, which were anesthetized immediately after capture, had plasma cortisol levels below 10 ng/mL, indicating that the experience of being captured and confined was stressful for all fish regardless of cage location. It is possible, then, that any number of factors could have contributed to the three instances of significantly elevated plasma cortisol levels. Such factors include (but may not be limited to) water temperature, noise and activity at the construction site, pile driving noise, confinement with fish of widely varying size and weight, and the cage handling and transport that occurred between exposure and necropsy.

Discussion

The study results indicate that exposure to underwater sound levels of up to 194 dB (cumulative SEL) did not result in immediate physical injuries to juvenile steelhead. The 194-dB cumulative SEL observed was substantially more than the 187-dB SEL threshold set by NMFS as indicative of potential harm to salmonids. However, fish could not be held for later observation (because of ongoing pile driving and high river temperatures), so it was not possible to assess the possibility that acoustic stress may have resulted in delayed effects as part of this experiment. However, research conducted by Ruggerone et al. (2008) using juvenile coho salmon found that there was no immediate or latent (10 to 19 days) mortality in fish exposed to 207 dB cumulative SEL from pile driving.

With the exception of fish captured individually and sacrificed immediately after capture at the hatchery, all exposed and control fish groups had elevated plasma cortisol levels. There was no pattern in elevated cortisol levels related to exposure to pile driving (i.e., received sound levels). Overall, the mean cortisol levels were lower from fish at the control site (52.2 ng/mL), but fish exposed to SEL levels below the 150-dB accumulation threshold at the 100- and 150-meter stations had the highest cortisol levels (113 and 108.6 ng/mL, respectively). This indicates that cortisol may not be a good indicator of stress related to pile driving, and is likely related to a variety of factors such as water temperature, handling, and confinement.

References

- Abbott, R. R., J. Reyff, and G. Marty. 2005. *Final Report: Monitoring of Effects of Conventional Pile Driving on Three Species of Fish*. Prepared for Manson Construction Company, Richmond, CA.
- California Department of Transportation. 2010a. *Mad River Bridges Replacement Project: Hydroacoustic Monitoring Report for Bridge Piles Driven in 2009*. March. Prepared by Illingworth & Rodkin, Petaluma, CA.

- . 2010b. *Necropsy and Histopathology of Steelhead Trout Exposed to Steel Pile Driving at the Mad River Bridges, U.S. Highway 101, July 2009*. March. Prepared by Gary D. Marty, DVM, Ph.D., Fish Pathology Services, Abbotsford, British Columbia, Canada.
- Govoni, J. J., L. R. Settle, and M. A. West. 2003. Trauma to Juvenile Pinfish and Spot Inflicted by Submarine Detonations. *Journal of Aquatic Animal Health* 156:111–119.
- Govoni, J. J., M. A. West, L. R. Settle, R. T. Lynch, and M. D. Greene. 2008. Effects of Underwater Explosions on Larval Fish: Implications for a Coastal Engineering Project. *Journal of Coastal Research* 24:228–233.
- Office of Laboratory Animal Welfare. 2002. Institutional Animal Care and Use Committee Guidebook. 2nd edition. Available: <<http://grants.nih.gov/grants/olaw/GuideBook.pdf>>, Accessed: February 5, 2010.
- Popper, A. N., M. B. Halvorsen, A. Kane, D. L. Miller, M. G. Smith, J. Song, P. Stein, and L. Wysocki. 2007. The Effect of High-Intensity, Low-Frequency Active Sonar on Rainbow Trout. *Journal of the Acoustic Society of America* 122:623–635.
- Popper, A. N., and M. C. Hastings. 2009. Review Paper: The Effect of Anthropogenic Sources of Sound on Fishes. *Journal of Fish Biology* 75:455–489.
- Ruggerone, G., S. Goodman, and R. Miner. 2008. *Behavioral Response and Survival of Juvenile Coho Salmon Exposed to Pile Driving Sounds*. Prepared for the Port of Seattle, Seattle, WA.