Pile Installation Demonstration Project

Analysis of Tissues of Fish Exposed to Pile Driving

AECOM

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1.0 Overview

The study involved examination of any fishes found at the surface or collected in a mid-water trawl as part of the Pile Installation Demonstration Project (PIDP) to determine the cause of mortality or injury. Tissue injury was determined using necropsy[1] methodology. Necropsy was performed on fishes collected to determine signs of tissue damage both externally and internally. The necropsy procedures (see the Appendix) were virtually identical to those developed in Dr. Popper's laboratory[2] during studies on the effects of pile driving on fishes (Halvorsen et al. 2011[3]). The full necropsy takes about 20-25 minutes, depending on fish size.

2.0 Fish Collection Procedures

During the one hour period prior to the start of pile driving, the area upstream and downstream of the pile location were surveyed to determine if there were any dead/dying fishes at the surface or in the mid-water column. During pile driving operations and for a period of one hour after pile driving stopped, continuous surveys were conducted to collect dead or injured fishes on the surface and in the mid-water column. See the Fish Monitoring Report for information on the collection methods and results.

Any fishes collected at the surface or in the mid-water trawl during the surveys were examined to determine whether they were injured or dead. Any fishes collected that were determined to be uninjured were to be returned to the river. Injured and dead fishes were examined for tissue damage, following the general sampling procedures defined for the project.

3.0 Necropsy Methodology

Necropsy was conducted using standard procedures as reported in the Appendix. All tissues were examined by a trained scientist, Dr. Brandon Casper. Dr. Casper has conducted fish necropsies following pile driving injury experiments for over three years and has been involved in developing the procedures described in the Appendix and the list of injuries described in Table 1. After external examination of the fishes to look for external bruising or other potential effects of pile driving, the fishes were dissected to reveal internal tissues using standard dissection techniques designed so as not to injure any internal tissues.

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[1] Necropsy is the animal equivalent of an autopsy.

[2] Dr. Casper was a postdoctoral fellow in the laboratory at the time of the referenced study and is co-author on the work. He has been "chief scientist" on all subsequent pile driving work in Dr. Popper's laboratory.

[3] Halvorsen, M.B., Casper, B.M., Woodley, C.M., Carlson, T.J., and Popper, A.N. (2011). Predicting and mitigating hydroacoustic impacts on fish from pile installations. NCHRP Research Results Digest 363, Project 25-28, National Cooperative Highway Research Program, Transportation Research Board, National Academy of Sciences, Washington, D.C. http://www.trb.org/Publications/Blurbs/166159.aspx

Table 1

Observed Injuries Associated with Exposure to Pile Driving

Injury Description	Biological Significance of Injury
Dead within 1 hour	Dead
Pericardial (heart) hemorrhage	Discrete organ, main body blood pump, bleeding from heart; decreased blood pressure
Hepatic (liver) hemorrhage	Discrete organ; bleeding from liver; decreased blood pressure
Renal (kidney) hemorrhage	Non-discrete spongy organ, held in place with membrane, bleeding; decreased blood pressure
Ruptured swim bladder	Lost ability to maintain buoyancy, sank to bottom; may affect hearing
Intestinal hemorrhage	Blood filling the abdominal cavity; decreasing blood pressure
Burst capillaries along body wall	Decreased ability to get blood to muscle; decreased blood pressure
Pericardial (heart) hematoma	Could decreased efficacy of heart
Intestinal hematoma	Major portal system, decreased amount of blood flow to the rest of body.
Renal (kidney) hematoma	Large amount of blood pooling in more severe cases
Body muscles hematoma	Could affect swimming ability
Swim bladder hematoma	Could affect ability to regulate buoyancy; could potentially affect hearing
Fat hematoma	Related to swim bladder, caused from swim bladder
Ovaries/testes hematoma	Potential short-term damage but potential long-term consequences for reproductive success
Blood spots on vent	Dilated capillaries near skin, respiratory acidosis, stress with a predisposition, or severe damage
Dorsal fin hematoma	Dilated capillaries near skin, respiratory acidosis, stress with a predisposition, or severe damage
Caudal fin hematoma	Dilated capillaries near skin, respiratory acidosis, or stress with a predisposition, or severe damage
Pelvic fin hematoma	Pelvic fin is near intestinal portal system
Pectoral fin hematoma	Pectoral fin is near the heart portal system
Anal fin hematoma	Dilated capillaries near skin, caused by respiratory acidosis, stress with a predisposition, or severe damage
Fully deflated swim bladder (no ruptures)	Negatively buoyant, which could be beneficial for less barotrauma, quick recovery by surface air gulp
Partially deflated swim bladder (no ruptures)	Negatively buoyant, which could be beneficial for less barotrauma, quick recovery by surface air gulp

Note: The injuries presented here are based on extensive studies on effects of pile driving injuries found in five different species of fishes by Dr. Popper and his colleagues (e.g., Halvorsen et al., 2011). This table gives injury type and a description of the injury and its potential biological significance.

The types of injuries that are potentially encountered as a result of pile driving are listed and described in Table 1. This table is from Halvorsen et al. (2011) and gives a range of injuries found in pile driving studies on five species of fishes, including several species that are the same as, or similar to, those found in the Hudson River (e.g., striped bass, hogchoker, and lake sturgeon). The list in the table identifies the most severe injury at the top, which often result in mortality shortly after exposure. The injuries listed towards the bottom of the table are likely not severe and are thought to be non-consequential in terms of affecting survival or from which fishes are likely to quickly recover (Halvorsen et al. 2011; Casper et al. 2012[4]).

4.0 Fish Collected

No fish were collected before, during or after pile driving of the five 4-foot piles installed during the PIDP. In addition, no fishes were collected on the surface or in the mid-water column during the surveys conducted prior to pile driving of the 10-foot and 8-foot piles.[5]

Two fishes were collected on May 14, 2012; one fish was collected during driving of a 10-foot pile and one fish after the pile driving. Both animals were at the surface and dead upon collection. One fish was collected during driving of the 8-foot pile. This fish was alive when collected, but in sufficiently bad condition to warrant sacrifice and examination. Details are provided below.

5.0 Details of the Necropsy of Fishes Collected During Pile Driving

Examples of normal tissues are shown in Figure 1 for comparison with damaged tissues shown in Figures 2 and 3. In Figure 1, photos A and B are from the white perch found dead during the driving of the 10' pile. The external damage encountered in this fish was only on one side, and so the opposite, normal, side is shown for purposes of comparison. Figure 2C shows a normal kidney from a striped bass.

5.1 Driving of 10-foot Pile (May 14, 2012)

A single dead tomcod, 4.7 cm in standard length,[6] was collected about 150 feet south of the pile driving barge during the driving of the 10-foot pile. The fish was floating on the surface dead. Dr. Casper performed a full necropsy, as described in the Appendix. The findings of the necropsy are shown in Table 2 with injuries displayed in Figure 3. There were no external injuries in this fish, but considerable internal bleeding that was seen to come from severe damage to the intestines which lie just below the swim bladder. No other sources of bleeding were found.

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[4] Casper, B. C., Popper, A. N., Matthews, F., Carlson, T. J., and Halvorsen, M. B. (2012). Recovery of barotrauma injuries in Chinook salmon, *Oncorhynchus tshawytscha* from exposure to pile driving sound. PLoS ONE, in press.

[5] The only exception were two found floating on the surface but which had clearly been dead for many hours, if not days. These fish were putrefied and/or showed substantial damage to fins and deep wounds that suggested that the dead fish had been fed upon by other creatures.

[6] Standard length is a general measure used for fishes that is the distance from the tip of the snout to the base of the caudal (tail) fin.

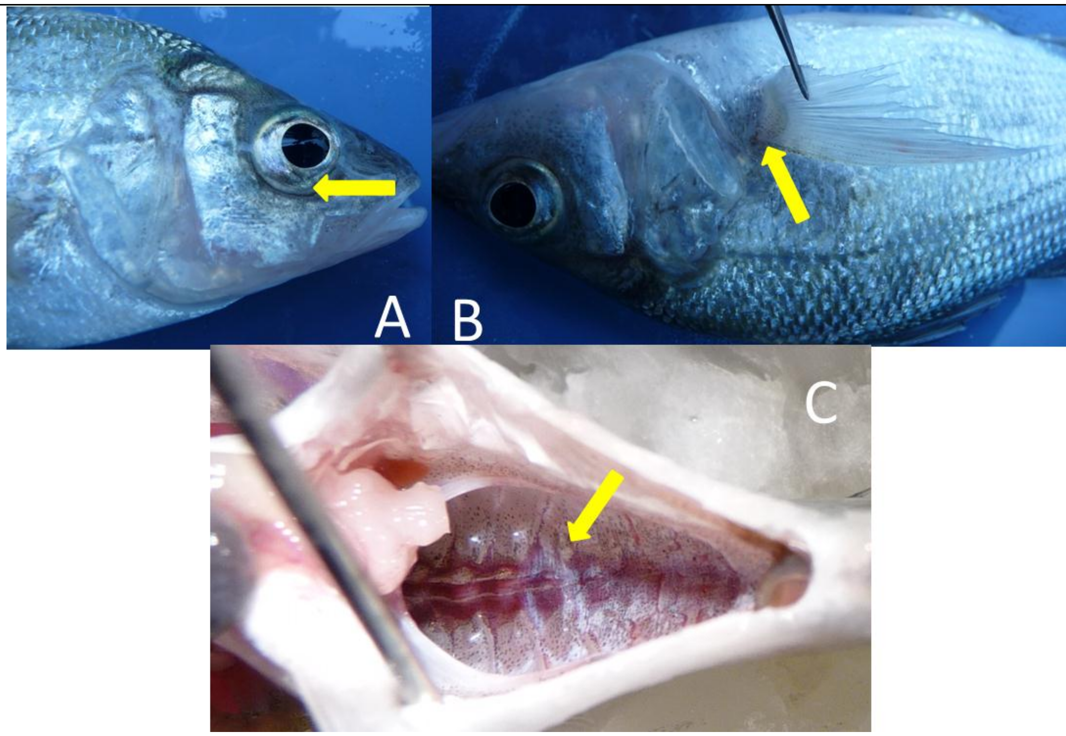

Figure 1 Examples of Healthy Structures
(Compare to the injured examples that are shown in Figures 2 and 3.)

Photos A and B are from the same white perch shown in Figure 2, but on the side of the animal that showed no injury. Photo A shows the normal right eye while Photo B shows the normal right pectoral fin.

Since there are no control fishes from the PIDP, Photo C gives an example of a healthy kidney in a striped bass from work in Dr. Popper's laboratory.

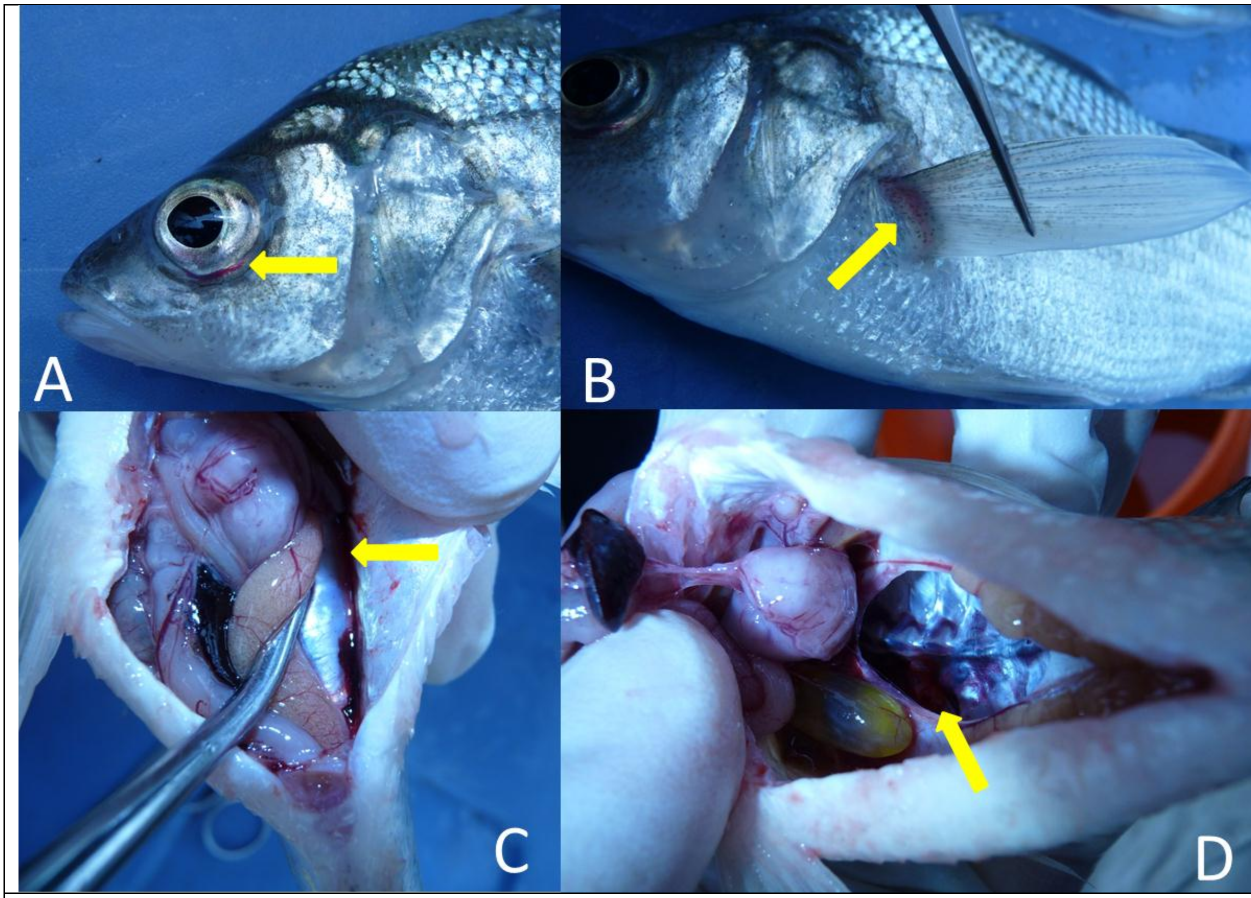

Figure 2 Examples of Injuries in the White Perch
(Are likely to have resulted from exposure to pile driving.)

Photos A and B are external anatomy photos while pictures C and D are internal anatomy photos in which the ventral portion of the fish has been cut open from anal pore to gill slits.

The orientation of Photo C is the dorsal end of the fish at the top of the picture and the orientation of Photo D is the dorsal end of the fish to the left.

The arrows on the photos point to:

A. Eye hemorrhaging.

B. Pectoral fin hematoma.

C. Blood pooling in the body cavity from kidney hemorrhaging.

D. The swim bladder has been cut open to reveal the kidney hemorrhaging.

Table 2

Injuries Observed in Fish During Tappan Zee PIDP
(Described in detail in Table 1)

Injury Description (see Halvorsen et al., 2011 for complete description of each injury)	Tomcod 5/14/ 2012 10-foot Pile	White Perch 5/14/2012 10-foot Pile	Tomcod 5/18/2012 8-foot Pile
Dead within 1 hour	X	X	Likely
Eye hemorrhage		X	
Pericardial (heart) hemorrhage			
Hepatic (liver) hemorrhage			X
Renal (kidney) hemorrhage		X	X
Ruptured swim bladder			X
Intestinal hemorrhage	X		X
Burst capillaries along body wall			
Pericardial (heart) hematoma			
Intestinal hematoma			
Renal (kidney) hematoma			X
Body muscles hematoma			
Swim bladder hematoma			
Fat hematoma			
Ovaries/testes hematoma			X
Blood spots on vent			
Dorsal fin hematoma			
Caudal fin hematoma			
Pelvic fin hematoma			
Pectoral fin hematoma		X	
Anal fin hematoma			
Fully deflated swim bladder (no ruptures)			
Partially deflated swim bladder (no ruptures)			
Note: An X in the column indicates that the injury was encountered in fish collected during the PIDP fish surveys.			

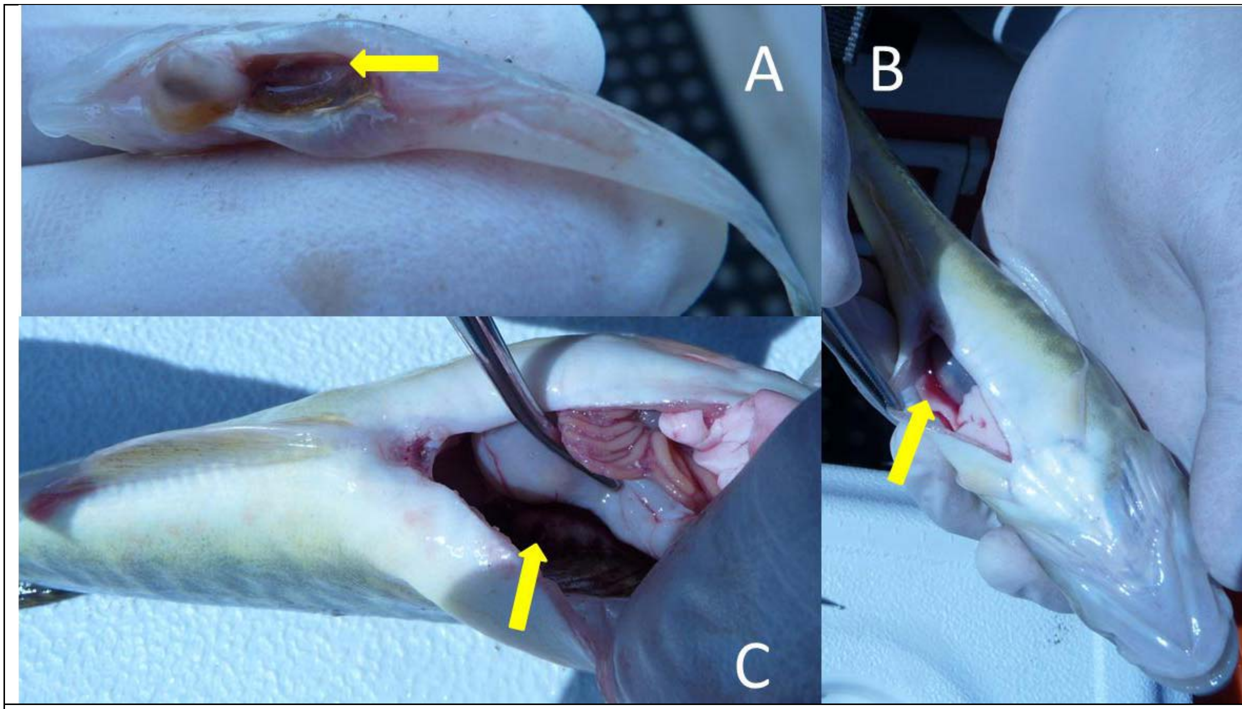

Figure 3 Examples of Barotrauma Injuries in the Two Tomcod

All three photos are the ventral sides of the fish as they were cut open from anal pore to gill slits.

In Photo A, the small tomcod has its dorsal end pointed to the left. The arrow is pointing towards an intestinal hemorrhage.

In Photo B, the larger tomcod has its dorsal end facing towards the bottom right corner. Example of blood pooling from a kidney hemorrhage are shown with the arrow.

In Photo C, the larger tomcod has its dorsal end facing towards the right. The arrow is pointing towards the hemorrhaging kidney.

A single white perch, 15.3 cm in standard length, was collected dead about 3,000 feet south of the pile driving barge during the survey period after the driving of the 10-foot pile had stopped. The fish was floating on the surface dead. Dr. Casper performed a full necropsy, as described in the Appendix. The findings of the necropsy are shown in Table 2, with injuries displayed in Figure 2. This fish had hematoma in the eye and the base of a fin (compare to the non-damaged side in Figure 1). Upon dissection, this fish was found to have severe internal bleeding that appeared to derive from the kidneys, structures which in fish, lie just dorsal to the swim bladder.

5.2 Driving of 8-foot Pile (May 18, 2012)

A single tomcod, 12.5 cm in standard length, was collected about 2,700 feet south of the pile driving barge, just below the current bridge during the final stages of driving of the 8-foot pile. The fish was on the surface and swimming on its side and, at times, upside down. It could not right itself in the water.

The fish was taken onto the boat and put into a bucket of river water where it was observed for about 10 minutes. During this time, the fish stayed at the surface, moving either upside down or, less frequently, on its side. In an attempt to help the fish recover, it was put into a screened enclosure along the side of the boat to give it a larger enclosure and fresh river water. The fish continued to show the same behavior and was continuously pushed against the rear of the cage due to the current in the river. Since this potentially caused more distress to the fish; it was gently netted and put into a large container on the boat. After about 20 minutes, it was decided that the fish was not recovering and the decision was made to sacrifice the animal using MS-222, an anesthetic that is veterinary approved for fish and other cold-blooded vertebrates.[7] The fish quickly succumbed to the anesthetic.

The fish was then removed from the water and Dr. Casper performed a full necropsy, as described in the Appendix. The findings of the necropsy are shown in Table 2 with examples of injuries shown in Figure 3. There were no external signs of damage, but considerable internal bleeding was seen upon dissection. Damage was found to the liver, kidney, intestines, and gonads. While the heart continued to beat, the opinion of both Dr. Casper and Dr. Popper was that this level of internal damage would have resulted in death soon after the fish was brought on board the boat.

6.0 Conclusions

No dead or injured fishes were found before, during, or after driving of any of the 4-foot piles. A total of three fishes were collected during the pile driving fish surveys for the PIDP. Two were collected during/after the driving of a 10-foot pile and one during the driving of the 8-foot pile.

All three fishes were found downstream from the pile driving operation. In the case of the tomcod found in distress during the driving of the 8-foot pile, the fish was within 150 feet of the pile. While it is impossible to know where the fish was when it was injured, there was a strong downstream current and it is very possible that the fish was very close to the pile driving operation, in a region that had the most intense sounds.

[7] See: http://onlinelibrary.wiley.com/doi/10.1111/j.1439-0426.2012.01950.x/abstract

The injuries encountered in all three fishes were identical to those found during extensive studies of the effects of pile driving on fishes conducted over the past several years in Dr. Popper's laboratory at the University of Maryland (see Halvorsen et al., 2011; Casper et al. 2012) and described in Table 1. Thus, it is reasonable to conclude that the injuries seen in the three fish collected during the PIDP were as a result of exposure to pile driving sounds.

ATTACHMENT A

Barotrauma Necropsy Protocol

External exam consisted of examination of the following tissues (modified from Halvorsen et al., 2011, Appendix D):

1. **Eyes** –signs of blood or presence of bubbles (embolism). Open the mouth and examine the underside of the eye through the roof of the mouth.
2. **Stomach protrusion** – Observed by looking into oral cavity.
3. **Gills –** Examine gill rakers, looking for bubbles along the filaments.
4. **Hemorrhage in pericardial cavity** –Examine for bubbles or pooled blood in the internal pericardial cavity by looking at the region behind the last gill raker.
5. **All Fins** – Examine each fin. Do this by spreading out. Look for bubbles inside the fin rays and at the base of the fin for bubbles or blood pooling.
6. **Anal pore** – Check for blood or bile coming out of the anal pore which would suggest an intestinal injury.

Internal Signs of Barotrauma

Make a ventral incision from the anal pore to the pericardial region to expose all internal organs mid-ventrally. Cut close to mid-ventral body wall so as not to cut into any internal tissues.

1. Before cutting into internal organs, look for any signs of blood that would suggest an internal injury.
2. **Liver** – Look for hemorrhaging on or around the liver; lift the liver and look underneath for damage.
3. **Heart** –Look for hematoma, hemorrhaging, and embolisms.
4. **Spleen** – Look for signs of hemorrhage.
5. **Fat** – Look for signs of hematoma.
6. **Gall Bladder** – Look for discoloration of internal fluid such as red or brown instead of bright green
7. **Pyloric Caecum** – Look for signs of damage.
8. **Intestine** – Inspect the entire intestine and associated vessels for damage.
9. **Gonads** – Examine testes or ovaries for any bruising or hemorrhaging.
10. **Swim bladder** – Move all the guts to the side: intestine, stomach, and any fat, to expose the entire swim bladder. Examine for perforations.
11. **Kidney** – Removal of the swim bladder to see the kidney. Examine for presence of any bubbles or hemorrhaging under the surface; and for damage to the vessels along the muscle walls.