*Final Draft*

# Effects of Seismic Surveys in Lake Sakakawea on Pallid Sturgeon and Other Key Fish Species

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## EXECUTIVE SUMMARY

The oil and gas industry is proposing to conduct seismic geophysical surveys within Lake Sakakawea, a reservoir in the Missouri River basin of central North Dakota. The Stony Creek 3D seismic survey is one such project proposed by Hess Corporation (Hess). If permitted, this project will include the use of airguns within specific areas of Lake Sakakawea. Because there is little information on the effects of seismic sound on fishes, Hess contracted CSA International, Inc. to design and conduct a study of the potential effects of seismic sound on selected fish species living in Lake Sakakawea.

This Study was designed to assist in determining acceptable sound pressure levels to mitigate possible impacts of such studies, and specifically by the Stony Creek 3D seismic survey proposed by Hess. The Study provides the quantified and statistically reliable data needed to evaluate the possible risks associated with the use of the specific airgun array proposed for the Hess project, including the specific sound pressure levels from that array, on species of concern (pallid sturgeon, paddlefish, and walleye) in Lake Sakakawea.

The Study consisted of exposing caged specimens of the three species to impulsive sound generated by a seismic airgun array using an experimental design that permitted a comprehensive statistical analysis of results. Using this design, fish were placed in cages at different distances from the airgun array in order to determine if there was a functional relationship between sound level and potential effects on body tissues such as the swim bladder and kidney. Control fish were treated identically to exposed fish except they received no seismic sound exposure when in the cages.

The single shot exposure paradigm used in this Study was selected because it was determined to be the best simulation of the proposed seismic survey strategy. That plan calls for the seismic vessel carrying the airgun to move along preplanned transects where a single shot would be generated by the airgun array at each preplanned shot point. After a shot is completed the vessel would move on the order of 100 m to the next location where another shot would be conducted. The distance traveled by the airgun vessel would, most likely, assure that if a fish were exposed to two shots, one shot would usually be much higher in energy than the other so that any observed effect could be assumed to be in consequence primarily of the higher energy exposure. Thus, in this experiment, it was decided that only a single shot would be appropriate to simulate the effective sound level to which fish would likely be exposed during the actual survey.

Fish were exposed to a single shot from the airgun array. The signal levels at the source, and at the cages in which fish were held, were continuously measured by a calibrated sound measurement system.

The sounds to which fish were exposed simulated the sound to which fishes would be exposed in an actual seismic survey. In such a survey, the seismic vessel moves 330 feet between shots. Thus, under normal circumstances, fishes are highly likely to encounter only one shot at maximum intensity; any other shots impinging on the fish would be substantially lower in intensity.

After the fish were exposed to the seismic array they were returned to the fish hatchery from which they were obtained. The fish were held for 7 days post-exposure and then euthanized (sacrificed), necropsied (autopsied), and examined for injuries that were potentially mortal.

The results from the Study showed that there was no mortality to any of the pallid sturgeon or paddlefish during exposure to the seismic airgun, even when the animals were in cages that were approximately 1 to 3 m from the guns where the exposed peak negative sound pressure level (Peak- SPL) was 224 dB re 1 μPa (205 dB re 1 $\mu Pa^2 \cdot s$ sound exposure level [SEL]). Moreover, there was no mortality over the 7-day post-exposure holding period.

Necropsy on the pallid sturgeon and paddlefish included examining the swim bladder and kidney for ruptures and hemorrhages. Damage to these tissues would potentially indicate a mortal injury, resulting in death in wild animals. Statistical analysis of the data showed that there were no differences in injury rates between exposed or control pallid sturgeon or paddlefish.

A similar study was conducted with both adult and young-of-year walleye. Unfortunately, most of the exposed and control fish died due to problems in handling the animals and issues with low oxygen in the transport tanks. Therefore it was not possible to do any statistical analysis on the data, thus the potential effects of seismic sound exposure on walleye could not be evaluated.

The results of the study for pallid sturgeon and paddlefish demonstrate that the probability of mortal injury in either species was the same for exposed fish as it was for control fish at least to 7 days post-exposure to sound from a seismic airgun array, the same size as that planned for the Stony Creek 3D seismic survey.

It is concluded that although each seismic survey differs in the size of the airgun array, operational water depths, and in the species potentially affected, the results from the Study indicate levels of impulsive seismic airgun sound that adult fish can be exposed to without immediate mortality. Thus, it is clear from the results of this Study that pallid sturgeon and paddlefish with body mass on the order of 200 to 400 g exposed to a received single impulse sound exposure level of 205 dB re 1 $\mu Pa^2 \cdot s$ did not die immediately or within 7 days of exposure, and that the probability of mortal injury did not differ between exposed and control fish.

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## TABLE OF CONTENTS

**Page**

## List of Tables

# List of Figures

# List of Figures
## (Continued)

| | |
|---|---|
| µPa | microPascal |
| $^{o}$C | degrees Celsius (Centigrade) |
| ADAMS | acoustic data acquisition system |
| AMAR | autonomous multichannel acoustic recorder |
| ANODEV | analysis of deviance |
| cm | centimeter |
| dB | decibel |
| $^{o}$F | degrees Fahrenheit |
| gal | gallon |
| GDNFH | Garrison Dam National Fish Hatchery |
| h | hour |
| km | kilometer |
| L | liter |
| m | meter |
| Peak- SPL | peak negative (rarefaction) sound pressure level |
| PIT | passive integrated transponder |
| ppm | parts per million |
| PVC | polyvinyl chloride |
| RMS | root-mean-square |
| SEL | sound exposure level |
| $SEL_{cum}$ | cumulative sound exposure level |
| $SEL_{ss}$ | single signal sound exposure level |
| SL | signal level |
| SPL | sound pressure level |
| USFWS | United States Fish and Wildlife Service |
| USGS | United States Geological Survey |
| YOY | young-of-year |

# 1.0 INTRODUCTION AND OVERVIEW

## 1.1 STATEMENT OF PROBLEM

The oil and gas industry is proposing to conduct additional seismic geophysical surveys within Lake Sakakawea, a reservoir in the Missouri River basin of central North Dakota. The Lake, which was established by the building of Garrison Dam in the 1950s, is the third largest man-made lake in the United States. Lake Sakakawea is inhabited by various fish species including the endangered pallid sturgeon (*Scaphirhynchus albus*[1]). Seismic exploration with the use of airguns has the potential to negatively impact fisheries resources (see **Section 1.2**). However, scientific information regarding the potential impacts of exposure to sounds produced by seismic airguns on pallid sturgeon (and other species) is very limited.

Hess Corporation (Hess) has proposed to conduct the Stony Creek 3D seismic survey, which includes the use of airguns within specific areas of Lake Sakakawea. Because little information is available on the effects of seismic sound on fishes, Hess contracted CSA International, Inc. to design and conduct a study of the potential effects of seismic sound on selected fish species living in Lake Sakakawea (hereinafter referred to as the Study).

A preliminary study conducted in Lake Sakakawea in 2009 found that the discharge of a larger seismic airgun array in close proximity may have lethal effects on young-of-year (YOY) pallid sturgeon (study discussed in **Section 1.2.1**). However, the 2009 study did not provide sufficient data on potential effects to determine if an airgun array of the size proposed for the Stony Creek 3D seismic survey would mortally impact fish. The 2009 study also did not provide enough data to determine whether mitigation measures are needed to protect the fish from exposure to sounds from a smaller airgun array or to enable development of mitigation measures to reduce or eliminate potential effects of the airgun array to be used during the Stony Creek 3D seismic survey on pallid sturgeons, if needed.

The fish exposure study described in this report was designed to assess the effect of exposure of pallid sturgeon, paddlefish, and walleye to seismic sound produced by the airgun array to be used by Hess in their planned Stony Creek 3D seismic survey.

To accomplish the goals of this project, the Study was designed to provide quantified and statistically reliable data to evaluate the risk of immediate and delayed mortality to larger pallid sturgeon and paddlefish exposed to impulsive sound produced by an airgun array of the same size as that to be used by Hess in their planned Stony Creek 3D seismic survey. The airgun array used in the Study (and to be used by Hess) is substantially smaller than that used in the 2009 study. This size difference, along with other factors discussed in **Section 1.2.1**, prevents using the results from the 2009 study to assess the potential effects on fish species of concern during the planned Stony Creek 3D seismic survey. The Study also was designed to assist the Army Corp of Engineers, the United States Fish and Wildlife Service (USFWS), and North Dakota Game and Fish in providing recommendations to minimize effects of these activities on pallid sturgeon and other fish species within Lake Sakakawea.

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[1] Scientific names for all species discussed in this report can be found in **Appendix A**. A glossary of scientific terms used in this report is in **Appendix B**.

The Study benefits future seismic activities through the generation of protocols and data that would enable the development of methodologies to reduce impacts from airguns if they were found to cause immediate or delayed mortality. Such mitigation methods might include careful selection of the characteristics of a seismic array and the number of acoustic pulses emitted at a given location.

A separate study was conducted during the same time period that investigated additional mitigation methods such as the use of sonar to determine if fish could be detected in the vicinity of a shotpoint prior to firing the airgun array (Hawkins, 2012).

## 1.2 EFFECTS OF SEISMIC AIRGUNS ON FISHES

### 1.2.1 Lake Sakakawea Fishes

Information regarding the potential impacts of seismic work on fish and fisheries in Lake Sakakawea is limited, as are data from effects of any impulsive sound source on fishes, including airguns and impact pile driving (reviewed in Popper and Hastings, 2009; Hawkins and Popper, 2012; Popper and Hawkins, 2012; see **Section 1.2.2**).

The only study on Lake Sakakawea fishes was a 2009 preliminary field investigation of effects on fishes from water guns and airguns conducted by Dr. Jackson Gross, then of the United States Geological Survey (USGS) Northern Rocky Mountain Science Center, along with the Missouri River Fish & Wildlife Conservation Office and Garrison Dam National Fish Hatchery (GDNFH). That study found that seismic activity from large volume airgun arrays has the potential to negatively impact pallid sturgeon. In that study, YOY pallid sturgeon (mean fork length = 150 mm) showed a 32% (62/198 fish) mortality by 14 days after exposure to a single pulse from a six-gun 20,647-$cm^3$ seismic airgun array compared with 2% (9/390 fish) mortality in control fish.

However, the usefulness of the 2009 work to informing the Study and the proposed Stony Creek 3D seismic survey is limited for several important reasons: 1) the airgun array in 2009 was twice the size of the one used in the Study (and planned for the Stony Creek 3D seismic survey) therefore the sound levels that fish were exposed to in 2009 were not comparable to current and proposed work; 2) there is no report of the 2009 study that can be used by regulators or investigators to compare with current work or to evaluate the 2009 study; 3) the size of the fish used in 2009 were substantially smaller than the fish used in the Study; and 4) there are no data from 2009 on the levels of signals received by the exposed fish that resulted in mortality.

### 1.2.2 Data from Scientific Literature

There have been only a few studies on effects of seismic airguns on fish and fisheries. These can be divided into studies that examined behavioral effects and studies that examined physiological effects (including mortality, the focus of the Study). In most studies, physiological effects are defined as any physiological change that may extend from “mild” injuries that result in minimal harm to the fish (e.g., minor bruising or bleeding externally), to “moderate” injuries that may or may not result in death at some time post-exposure (e.g., internal hemorrhage), to “mortal” injuries, which always result in death either immediately or at some time post-exposure (e.g., rupture of the swim bladder, hemorrhage of the kidneys or gonads) (Halvorsen et al., 2011, 2012a). Based on limited airgun data and studies on other impulsive sound sources, it is likely that physiological effects will occur only when fishes are exposed to very high sound levels from airguns (Popper and Hastings, 2009; Casper et al., 2012;

Halvorsen et al., 2012a,b; Hawkins and Popper, 2012). These physiological effects may take place within a few meters to tens of meters from a seismic source, depending on the configuration and size of airgun in the airgun array that constitutes the seismic source.

More complete reviews of the effects of man-made sounds on fishes can be found in Popper and Hastings (2009), Hawkins and Popper (2012), and in chapters in Popper and Hawkins (2012). Additional references on fish use of sound and related topics can be found in review papers in Webb et al. (2008) and in reviews by Popper and Hastings (2009) and Hawkins and Popper (2012).

#### *1.2.2.1 Behavioral Effects*

Several studies (e.g., Engås et al., 1996; Engås and Løkkeborg, 2002; Slotte et al., 2004; Løkkeborg et al., 2012a, b) have examined behavior of wild fishes in the ocean during exposure to large towed airgun arrays.[2] However, no work was done on species related to those in Lake Sakakawea, and the studies do not inform this fish exposure study or seismic studies in Lake Sakakawea.

The one potentially relevant study may be an investigation of the behavior of wild fish in a riverine environment where the water depth was closer to that of Lake Sakakawea. In that study, Jorgensen and Gyselman (2009; also Cott et al., 2012) used sonar to determine the behavioral responses of wild free-swimming fishes to noise from seismic airguns (e.g., swimming direction or speed) in the Mackenzie River (Northwest Territories, Canada). Fishes did not exhibit a noticeable response even when sound exposure levels (single discharge) were on the order of 175 dB re 1 $\mu Pa^2 \cdot s$ and peak sound pressure levels were greater than 200 dB re 1 µPa. While the species were very different from those in Lake Sakakawea, they do suggest that some fishes will not show changes in movement patters in response to moderately loud airgun sounds in a riverine environment.

#### *1.2.2.2 Physiological Effects*

A small number of studies examined physiological effects of exposure to seismic sounds on fishes. In all cases these were done with fishes in cages. However, unlike behavioral studies, physiological studies on caged fish are largely valid, but the assumption is made that the test fishes physiologically represent wild fishes and that exposure scenarios are similar, i.e., wild fish do not move from the site of the seismic activity.

To date, there are few data to document that fishes near a firing airgun are harmed physiologically, or that there are mortal effects. The only documented effects were found in a study by McCauley et al. (2003) who determined the effects of airgun exposure (received level 187 dB 1 $\mu Pa^2 \cdot s$ cumulative sound exposure level [SEL]; 226 dB re 1 µPa peak sound pressure level [SPL]) on the sensory hair cells of the ears of the Australian pink snapper (*Pagrus auratus*). Damage was found within 18 h post-exposure and was extensive when fish were examined 58 days post-exposure compared to controls. McCauley et al. (2003) did not examine any other body tissues to determine potential effects.

In another study that examined both the effects on the sensory cells of the ear and hearing, Popper et al. (2005) investigated the effects of exposure to an airgun array (received level 177 dB re 1 $\mu Pa^2 \cdot s$ cumulative SEL; 207 dB re 1 µPa peak SPL) on three fish species in the Mackenzie River Delta: northern

[2] Caged fish studies (e.g., Fewtrell and McCauley, 2012) are not considered here because the behavior of fishes in cages is substantially different from behavior of unrestrained animals. Thus, information from caged studies does not inform understanding of potential behavioral changes in wild fish in Lake Sakakawea (Hawkins and Popper, 2012).

pike (*Esox lucius*), broad whitefish (*Coregonus nasus*), and lake chub (*Couesius plumbeus*) (see also Cott et al., 2012). Fish were exposed to 5 or 20 airgun shots, while controls were placed in the same cage but without airgun exposure. Hearing in both exposed and control fish were then tested. Some loss of hearing was found in northern pike and lake chub, but not in broad whitefish, and both affected species showed complete recovery from hearing loss within 18 h of exposure. Morphological analysis showed no damage to inner ear sensory hair cells in any of the species (Song et al., 2008). While the study did not systematically investigate possible effects on other body tissues, Popper et al. (2005) reported that if any other tissues were affected such effects were, at most, minor and did not lead to swim bladder damage or hemorrhaging.

The overall outcome of these studies was that there were no mortal injuries to fishes as a result of airgun exposure, some small hearing loss occurred in some species, and damage to sensory tissues occurred in only one species. In a number of these studies the sound levels to which the fish were exposed equaled or exceeded the exposure levels in the Study.

#### *1.2.2.3 Eggs and Larvae*

There has been particular concern over the impact of seismic airguns on the eggs and larvae of fishes because of their small size and physical fragility. However, there are very few relevant data. Kostyvchenko (1973) and Booman et al. (1996) found indications of effects on fish eggs when exposed to an airgun shot at a close distance (the sound level at the eggs were not indicated). Saetre and Ona (1996) observed effects of seismic signals on fish larvae, but again there was no information on sound levels. Dalen and Knutsen (1987) concluded that so few eggs and fry were present within the very small danger zone around the airgun that the damage caused would have no negative consequences for fish stocks.

In a recent study using sounds from another intense impulsive sound source, pile driving, Bolle et al. (2012) found no mortality in larval common sole (*Solea solea*) that could be attributed to sound exposure. The levels of sound exposure varied across these studies, as did the species of fish. As a consequence, no general conclusions about the probably level of exposure at the threshold of physiological injury are possible, though there is little evidence of mortal injury.

## 1.3 OVERVIEW OF EXPERIMENTAL APPROACH

The overall final experimental approach used in the Study was to expose pallid sturgeon, paddlefish, and walleye to a seismic airgun array and determine whether exposure caused immediate mortality or injuries that could result in delayed mortality. More specifically, the three species were placed in cages in Lake Sakakawea, exposed to sound, removed from the cages, and returned to holding tanks in GDNFH. Fish were then sacrificed on day 7 following exposure and necropsied to determine if there were potentially mortal injuries. Fish that died, or were determined to be dying, before the 7-day holding period were sacrificed and necropsied immediately. Data are presented for pallid sturgeon and paddlefish. For a variety of reasons discussed below, data from walleye were not deemed useable.

Samples of fish were placed in cages located at different distances from the seismic airgun array configured to have the same output as the array to be used for the planned Stony Creek 3D seismic survey. Thus, fish in the cages at different distances from the source received a different exposure sound level when the airgun was fired. With this configuration, the fish in cages close to the source received a high level of sound exposure and those more distant received a significantly lower level of sound.  Controls were subject to the identical treatment as the exposed animals but without exposure to sound generated by the seismic airgun.

The single shot exposure paradigm used in this Study was selected because it was determined to be the best simulation of the proposed Stony Creek 3D seismic survey strategy.  That plan calls for the seismic vessel carrying the airgun to move along preplanned transects where a single shot would be generated by the airgun array at each preplanned shot point.  After a shot is completed the vessel would move on the order of 100 m to the next location where another shot would be conducted.  The distance traveled by the airgun vessel would, most likely, assure that if a fish were exposed to two shots, one shot would usually be much higher in energy than the other so that any observed effect could be assumed to be in consequence primarily of the higher energy exposure.  Thus, in the this experiment, it was decided that only a single shot would be appropriate to simulate the effective sound level to which fish would likely be exposed during the actual seismic survey.

# 2.0 METHODS

## 2.1 STUDY SITE LOCATION

The study site was located on the west side of Lake Sakakawea State Park (**Figures 1** and **2**), which is on the south side of the eastern end of Lake Sakakawea, North Dakota, near Park, North Dakota). The GDNFH is located approximately 1.61 km east-southeast of Lake Sakakawea State Park (**Figure 2**).

### 2.1.1 Site Selection Criteria

The fish exposure study site, which was in Lake Sakakawea State Park, is shown in **Figure 3**. The reasons for choosing the site for the exposure tests included the following requirements:

- keeping the fish transport distance as short as possible from the GDNFH to the experiment site to ensure the least possible stress to the fish;
- having an equipment staging area as close as possible to the experiment site;
- the availability of a boat ramp that could accommodate the support vessels and launching of the airgun barge;
- a secure area for the test equipment and vessels;
- a protected area from wind and waves;
- an area suitable for personnel and equipment logistics; and
- an area with low likelihood of the occurrence of wild pallid sturgeon so as not to potentially impact non-experimental animals.

The site for the Study was not in the same area as the proposed Stony Creek 3D seismic survey because that site was not conducive to conducting the Study. In particular, access to the hatchery for fish maintenance and data analysis and areas for personnel and equipment were imperative to carry out the Study.

### 2.1.2 Depth Profile

Prior to conducting the Study and selecting the precise site in which to expose fish, the only available water depth information was from a 2005 Navionics survey[3] (**Figure 4**). In selecting a site for exposure it was important to find an area where the water depths were near constant to eliminate, as much as possible, non-uniform acoustic propagation along the length of the study site due to differing water depths. Because the bays that branch from the main body of the reservoir are similar to canyons filled with water, it became a challenge to find such an area. However, the site chosen did provide, as much as possible, the desired water depth profile (**Figures 3** and **4**).

[3] The Navionics bathymetry data was acquired from a Windows PC software titled “PC App” provided by Navionics. The North Dakota Lakes region was used for this project.


Figure 1. Study site location, Lake Sakakawea, North Dakota.


Figure 2. Lake Sakakawea State Park (study site) and Garrison Dam National Fish Hatchery.


Figure 3. Study site location.

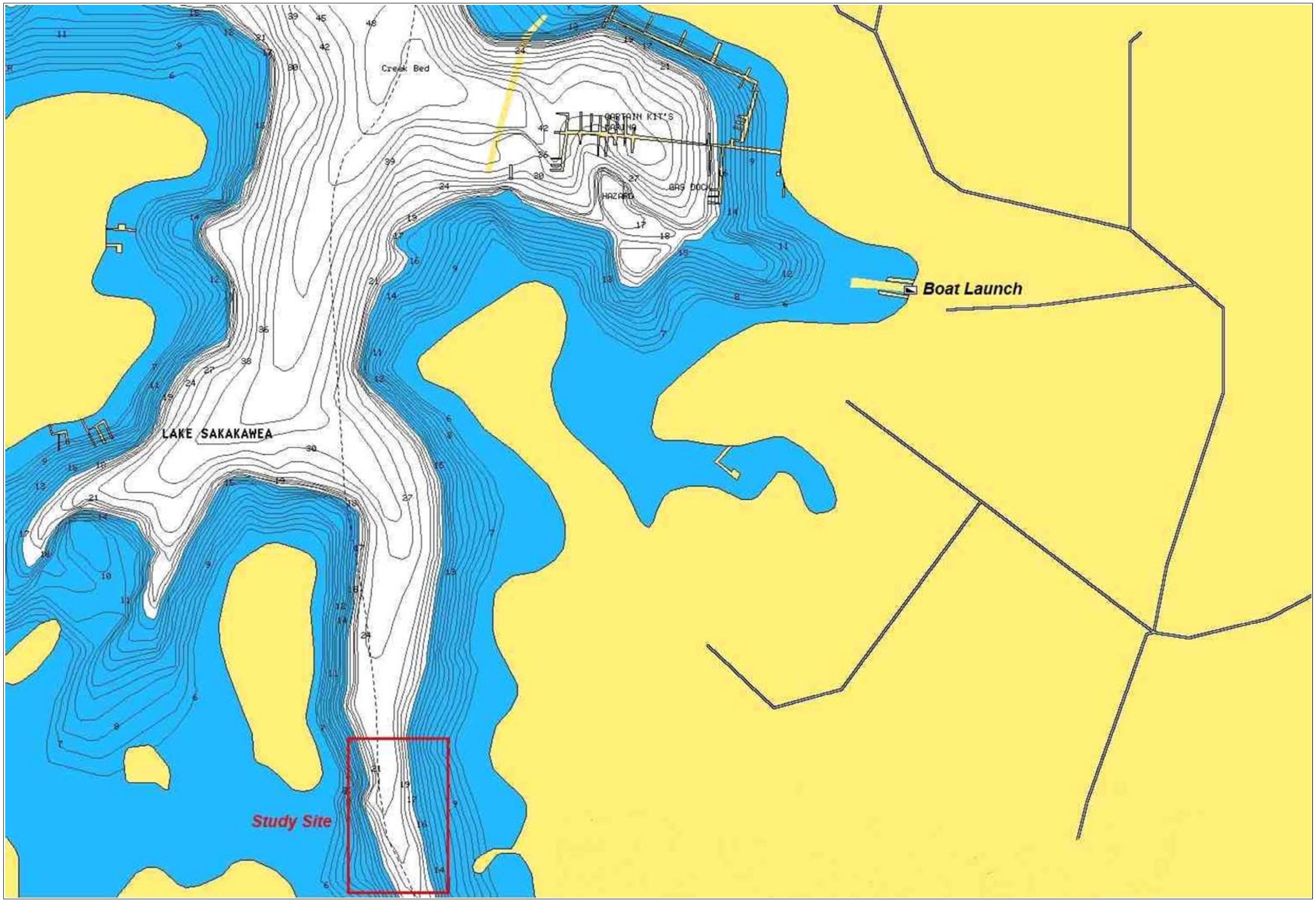



Figure 4. Bathymetry contours at the Study site, Navionics 2005.

## 2.2 FISHES

Three-year-old pallid sturgeon (sizes for all fish are shown in **Table 1**) and two-year-old paddlefish were hatched and reared at GDNFH specifically for this Study. The adult walleye were taken live from Lake Sakakawea in May 2012 to obtain brood stock and held at the GDNFH after use for the purpose of this Study. YOY walleye were hatched and reared at the GDNFH. Pictures of the three species are shown in **Figure 5**. Fish actually used in the Study were all within one standard deviation from the mean length of the population for the individual species available to the investigators (**Table 1**).

Table 1. Number of fish of each species exposed or used as controls in the fish exposure study. (Fork length was used for pallid sturgeon and paddlefish, total length for walleye.)

| Species | | Number of Fish Used (exposed and controls) | Mean Fish Length (mm ± SD) | Mean Fish Weight (g ± SD) |
|---|---|---|---|---|
| Pallid sturgeon | | 90 | 414 ± 25 | 224 ± 63 |
| Paddlefish | | 71 | 468 ± 17 | 352 ± 44 |
| Walleye | Adult | 90 | 493 ± 23 | 1,059 ± 188 |
| | Young-of-year | 90 | 157 ± 10 | 33 ± 7 |

SD = standard deviation.

a)

b)

c)

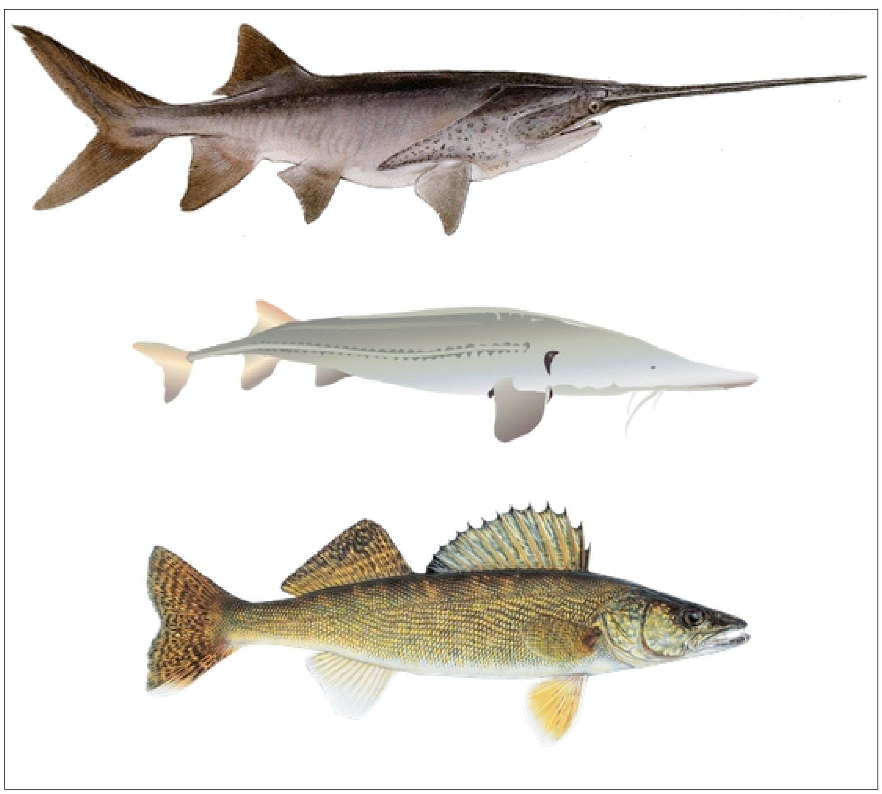


Figure 5. Illustrations of a) an American paddlefish, b) pallid sturgeon, and c) walleye (From: Texas Parks and Wildlife Department, 2012; University of Maryland Center for Environmental Science, 2012; Fish Index, 2012; respectively).

At the hatchery, pallid sturgeon and paddlefish were held together in 1.8 m diameter circular, black fiberglass tanks while adult walleye were held in 1.8 m wide concrete raceways. YOY walleye were reared in outdoor ponds and transferred to a cement raceway in the hatchery building approximately 5 days before sound exposure. Water for the hatchery was provided by ambient lake water with a hatchery temperature of 14 $^{o}$C during the experimental weeks of September 6 to 23.

### 2.2.1 Fish Identification

Adult fish used in this Study were individually marked on September 7, 2012, approximately 6 to 8 days before sound exposure. All fish were handled the same way and without sedation. Each member of the tagging teams was supervised by someone with expertise in the process, and it was noted which fish were tagged by each team.

Tagging involved fish being taken individually from holding tanks with a dip net, measured (fork length for pallid sturgeon and paddlefish, total length for walleye), tagged, and placed in a separate tank that held only tagged fish. Tag numbers were recorded along with fish length.[4] Pallid sturgeon were implanted with a passive integrated transponder (PIT) tag while paddle fish and the adult walleye received Floy T-bar anchor tags (Floy Tag, Seattle, Washington; www.floytag.com). Each individual tag was placed in the dorsal musculature posterior, lateral to the dorsal fin. **Figure 6** shows photographs of the two types of tags used.

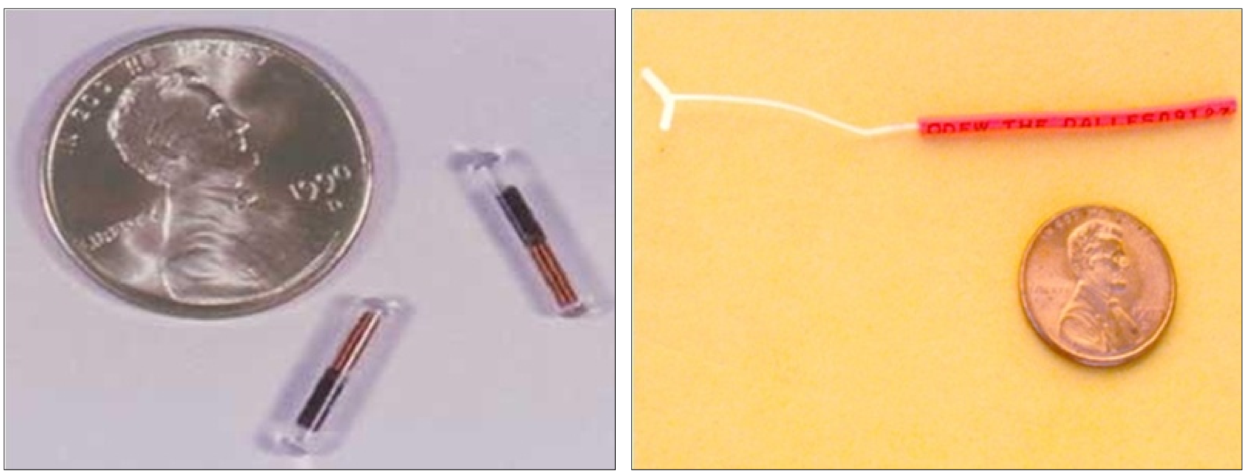


Figure 6. Passive integrated transponder (left) used to tag pallid sturgeon and Floy T-bar anchor tag (right) used to tag paddlefish and adult walleye.

YOY walleye were marked on September 15, 2012, the day of their exposure. Marking was done by fin clipping. Each fin was given a number, and non-repeating number was created for each fish by clipping a combination of fins.

[4] Refer to **Appendix B** for examples of data sheets used to record information throughout the study.

## 2.3 FISH CAGES

Fish exposure cages for pallid sturgeon, paddlefish, and adult walleye (**Figure 7**) were constructed of 2.54-cm square braided knotless mesh mounted in a frame constructed of 2.54-cm polyvinyl chloride (PVC) pipe. The mesh cages 1 m high x 1.5 m wide were designed to: 1) keep all fish as close as possible to the center of the cage so that all were exposed to the same signal level; 2) provide ample swimming space for up to five fish per cage (though fewer were always used); 3) reduce the risk of entanglement or injury to fish from the mesh or hard frame; and 4) allow for continuous swimming with an octagonal-shaped cage (no right angles) because paddlefish and sturgeon have rather inflexible bodies and cannot easily turn. Both species also require moving water and/or the ability to swim continuously so as to provide movement of water across gill membranes for respiration.

Figure 7. Octagonal-shaped fish cages.

Smaller cages 0.06 $m^3$ were built for the YOY walleye to prevent direct contact with the adult walleye during exposure to sound. The YOY walleye cages were constructed with 0.3-cm knotless mesh and were suspended within a frame constructed of 2.54-cm PVC pipe. During sound exposure the YOY walleye cages were attached to the top of the larger adult walleye cages by means of carabineers.

## 2.4 FISH TRANSPORT TO EXPERIMENT SITE

Fish were transported from the hatchery to the marina boat ramp with a fish transport trailer carrying two 2,180 liter (L) circular insulated fiberglass tanks (**Figure 8**). The tanks were filled at the hatchery with water pumped from the lake just prior to the loading fish. In addition, immediately prior to transport of fish back to the hatchery, the tanks were filled with water pumped directly from the lake. The fish transport tanks were designed with equipment to keep the water aerated with oxygen and circulating.


Figure 8. Fish transport truck with tanks.

For pallid sturgeon and paddlefish, one tank was used to transport fish to the study site and the other back to the hatchery. This protocol prevented mixing of exposed unused fish in case not all fish taken to the site were used for the Study.

Fish were placed in the transport tank by dip netting from the holding tanks at the GDNFH. As the fish were moved to the tank, they were again measured and tags read. If the fish were within the size range to be used for that species (**Table 1**), they were placed in the transport tank. Otherwise, they were returned to the holding tank. During the sorting period care was taken to ensure proper aeration and water quality in the holding tanks on the trailer.

Once the transport truck arrived at the marina boat ramp the fish were transferred by dip net into one of three rectangular aluminum cattle troughs on the pontoon barge (**Figure 9**) used to transport fish to the test site (**Figure 3**). The troughs were covered with tarps to prevent direct light from contacting the water surface and to help control water temperature. One trough had a capacity of 757 L and the other two 379 L. Water depth in the troughs was maintained at 0.3 m.

Figure 9. Fish pontoon barge used to move fish from the boat launch site to near the test site.

The barge troughs were initially filled with water from the transport trailer tanks to minimize risk of temperature shock because the surface water temperature of Lake Sakakawea was 20 $^{o}$C whereas the hatchery water temperature was 14 $^{o}$C. Once fish were in pontoon barge troughs and moved to deeper water, a sump pump was lowered into the lake and lake water slowly pumped into the troughs until the water in the troughs was completely replaced by lake water, a process that took about an hour. Water temperature and dissolved oxygen levels were monitored during the temperature acclimation process and recorded approximately every 30 min.

All exposed and unexposed pallid sturgeon and paddlefish were held on the pontoon barge for the duration of the experiment except when they were transported to the test cages for exposure to sound. At the completion of all sound exposures in a given day, the pontoon barge returned the fish to the boat ramp. The fish were then transferred to the empty tank on the haul trailer by dip netting and immediately returned to the hatchery. Sturgeon and paddlefish were on the pontoon barge for 4.25 to 4.5 h (the time from haul trailer back to haul trailer). While fish remained in the hatchery haul trailer for 0.5 to 1 h, the actual ride from the hatchery to the boat ramp did not exceed 10 to 15 min.

While pallid sturgeon and paddlefish were tested on separate days, both adult and YOY walleye were tested simultaneously. This was done because the adult walleye were too large for all to be transported in one transport trailer tank as had been done for the other species. Thus, the adult and YOY walleye divided into two groups, each of which was placed into one of the transport tanks. A total of 92 walleye (53 adults and 39 juveniles) were placed in one tank while the remaining fish (62 adults and 62 juveniles) were transported in the second tank. All fish from one tank were then transferred to the pontoon barge, tested, and then returned to the boat ramp and placed in the same tank they had been in during the transport to the boat launch. The fish from the second tank were then placed on the pontoon barge and tested.

During the walleye study, temperature and dissolved oxygen were not monitored in the tanks on the haul trailer during the experiments because all available staff were on the fish transport barge or otherwise assisting with exposure of test fish to sound. When the first load of exposed fish were returned to the trailer the dissolved oxygen in the tank holding the unexposed fish was found to be supersaturated with oxygen at a concentration of 22 ppm. When the second group was returned to the boat launch following exposure to sound the concentration of oxygen in the tank holding the first group of fish was found be very low (3 ppm) with many fish in the tank having suffocated and were dead or moribund.

Once back at the hatchery, sturgeon and paddlefish were transferred to 1.83 m x 2.4 m oval black fiberglass tanks (11.5 $m^3$), and walleye were placed back in the concrete raceway and separated from other fish by a divider. Fish were monitored every 12 h for dissolved oxygen and fish mortality while being held. Feeding of the test fish was stopped the day before tagging and was not resumed for the duration of the Study.

## 2.5 EXPERIMENT

### 2.5.1 Fish Cage Location

Five cages were positioned at various distances from the array (**Figures 10** and **11**). In addition, a sixth control cage was placed about 150 m south of the array. Fish placed in the control cage were treated identically to the fish in the sound exposure cages, except that the airgun array was not fired when control fish were in the water (**Figure 12**).

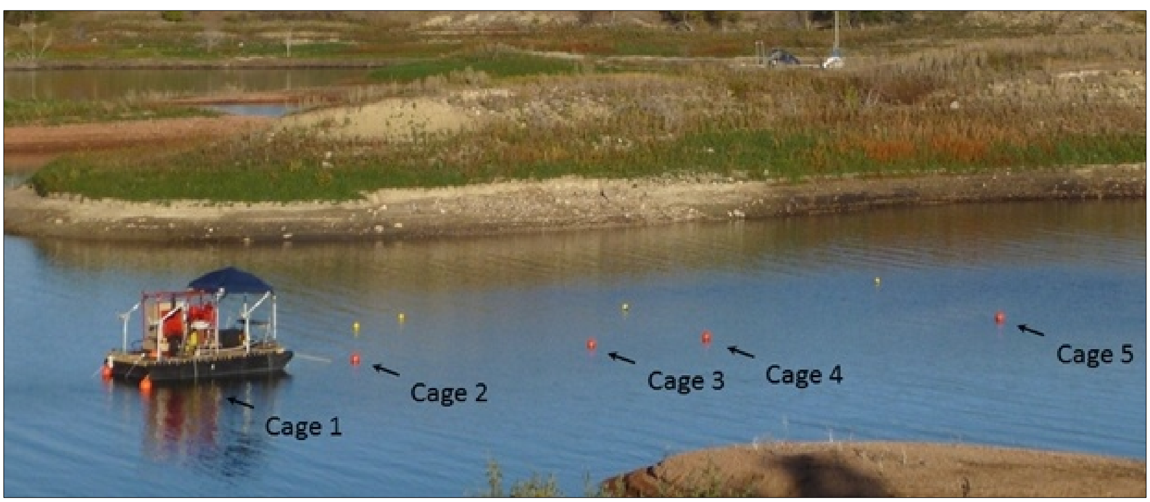



Figure 10. Airgun barge and fish exposure cage locations in the test area of Lake Sakakawea. Red floats indicate cage and autonomous multichannel acoustic recorder (AMAR) locations (see **Section 2.7.1**). Yellow floats are surface floats used for AMAR retrieval (they do not indicate the location of the AMARs). Airguns were hung from davits near the corners of the barge. Control cage is not shown in this figure, but it would be to the left (south) of the airgun barge.

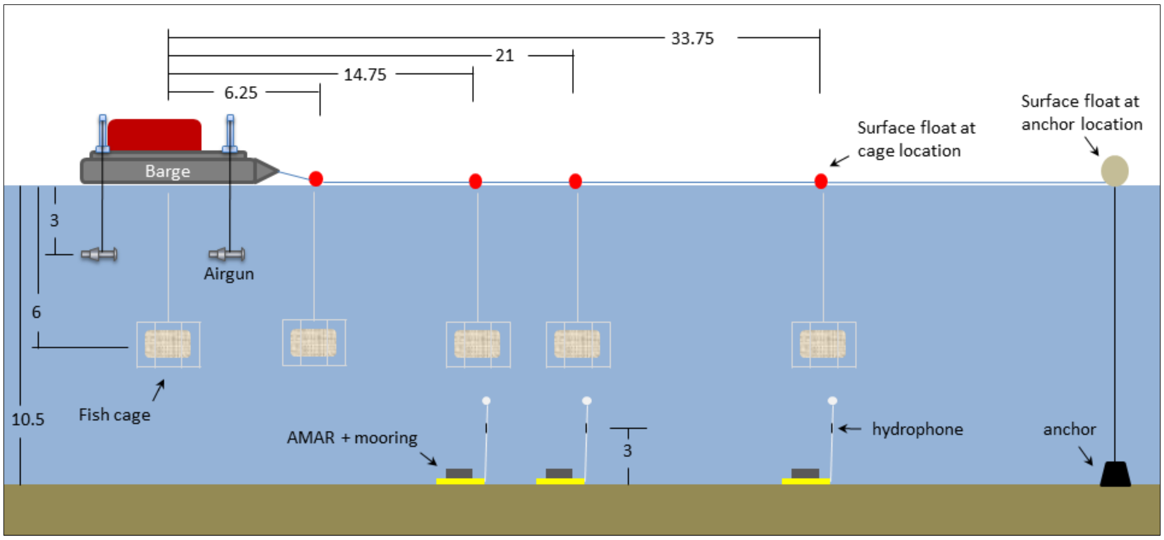



Figure 11. Location of the five exposure cages relative to the airgun barge (upper left) and the airgun (just below the barge). Distances in meters. Figure shows exposure cages at a depth of 6 m, for pallid sturgeon and walleye. For paddlefish, the exposure cages were shallower than the airgun array.


Figure 12. Study site in Lake Sakakawea. The location of the airgun barge is indicated by the square and the locations of the cages, to the north, are shown by circles. The site for the control cage is shown about 150 m south of the airgun barge. The site where the fish were transferred from the pontoon barge to the aluminum boats for loading in the cages is also shown.

The five treatment cages were all at the same water depth, which was the same for pallid sturgeon and walleye but was shallower for paddlefish (**Table 2**). The cages were distributed horizontally as described in detail in **Section 2.3.3** (**Figure 11**). The control cage was 6.4 m deep for all species.

Table 2. Fish exposure information.

| Species | | Number of Replicates | Number of Fish Exposed Per Cage | Cage Depth* | Time from Placement on Pontoon Boat to Return to Haul Trailer |
|---|---|---|---|---|---|
| Pallid sturgeon | | 5 | 3 | 6 m | 4.33 h |
| Paddlefish | | 3 | 4 | 2 m | 4.25 h |
| Walleye | Adult | 5 | 3 | 6 m | 7 h |
| | Young-of-year | 5 | 3 | 6 m | 7 h |

*Cage depth is the water depth of the cage measured from the vertical center of the cage.

### 2.5.2 Exposure Methodology

The pontoon barge, with test fish in the troughs, was moved to the general area of the test site (**Figure 12**). Once the boat reached that site, the fish were dip netted from the troughs and transferred to 190 L containers (totes) of lake water aboard aluminum flat bottom boats and transported to the exposure cages. Water in the totes was actively aerated by ambient air and replaced approximately every other trip to an exposure location. Dissolved oxygen and water temperatures were monitored and recorded every trip prior to fish being delivered for exposure to ensure that water quality was safe for fish health.

During the transfer process each fish was individually identified (using the tags) and assigned to a randomly pre-assigned test cage (located at different distances from the airgun). Each location was represented by a different tote. The aluminum boat then transported the fish to the fish cages. The exposure location (and tote) for each fish was recorded along with fish identification.

As soon as the aluminum boat arrived at a test cage, fish were individually lifted by dip net from the tote assigned to that cage and placed into the cage. Once loaded with fish, the cage was lowered to the depth designated for the particular species (**Table 2**) and the aluminum boat moved to a safe distance away from the airgun barge, at which time the compressor was activated and the airgun charged to its operating pressure.

Sixty seconds after operating air pressure was reached, the airgun array was triggered and the fish exposed to the seismic sounds. The aluminum boat then returned to the cage location and the cage was raised to the surface. The fish were removed from the cage using a dip net and put into an empty tote, taken to the pontoon barge, and then placed into a receiving trough. The average time required to place the fish into a tote, expose the fish to sound, recover the exposed fish, and return the fish to the recovery tough on the pontoon barge was about 10 min.

Paddlefish were exposed at a depth of 2 m, which was approximately 1 m above the depth of the airgun array. Pallid sturgeon and walleye were exposed at a depth of 6 m, which was approximately 3 m below the depth of the airgun array. The exposure depth for the paddlefish was selected to better represent the depths at which they might be exposed to seismic sound. Paddlefish are surface-oriented filter feeders with behavior that is quite different from that of the deeper living, more bottom oriented pallid sturgeon and walleye.

### 2.5.3 Experimental Design

During testing, one cage at a time was filled with three or four fish of one species, immediately lowered to the specified depth (**Table 2**), exposed to one shot from the airgun array, and then returned to the surface. (No sound was produced when control fish were in the cage, but otherwise treatment of the controls was *identical* to that of the exposed fish.) By exposing only one cage at a time it was possible to ensure that all fish were treated consistently and all spent the same amount of time at depth before being exposed to airgun sounds. It should be noted that the physiological condition of fish at the time of exposure, including whether the swim bladder was full at depth, was unknown other than that the fish were active and appeared healthy before being lowered to depth.

Each experiment was conducted using a randomized block design to ensure that equal data would be randomly collected from each block of exposure locations for a given experiment.[5] A block is defined as a single replicate from each of the six exposure locations. In other words, a block consisted of exposing a set of fish in cages 1 to 5 plus the control in a random sequence. The random order of exposure location was determined prior to testing using a random number generator for each replicate block of exposures. Once a single block was finished, another block was run, using a different random sequence. Thus, a typical first block might be in the sequence "3, 1, 4, control, 5, 2," while the next block might be "control, 5, 3, 4, 1, 2." The number of replicates (blocks) and fish exposed in each cage is indicated in **Table 2**.

## 2.6 AIRGUN BARGE

The airgun barge (**Figure 13**) was constructed on site and outfitted with four Bolt Technologies Incorporated (Norwalk, Connecticut) Long Life airguns. Three airguns were 2,294 $cm^3$ while one was 3,277 $cm^3$, totaling 10,160 $cm^3$.


Figure 13. Barge from which airguns were lowered with davits and positioned under the boat.

[5] The actual sequence of exposures in each block are found in **Table 4**.

The barge was constructed of high density polyethylene foam-filled pontoon floats and designed and constructed to support the 2,722 kg 100 cfm @ 34,473 kPa air compressor (Stark Industries, Houston, Texas) and 372 kg weight of the four airguns. The pontoon floats were bolted together, steel box beams were added, and a thick plywood deck was bolted to the top. Davits were mounted in a rectangular configuration, 2.75 m wide by 3.7 m long. The four airguns were raised and lowered to a depth of 3 m using hand winches mounted on the davits. The airguns were operated using a Hot Shot control module (Real Time Systems, Houston, Texas).

## 2.7 ACOUSTIC METHODOLOGY

A brief overview of the sound measuring methodology is presented here. A synopsis of sound levels at each recording location is shown in **Table 3**. Details of equipment, processing methods, and individual shot metrics are presented in **Appendix C**.

Table 3. Average sound levels measured at the different cages (see **Appendix C** for precise values plus standard deviation between shots). Cage 1 to 5 sound levels are those resulting from airgun shots. The ambient noise level in the control cage when fish were present, but without airgun shots, is also shown. These represent ambient noise levels in the lake at the cage location.

| Cage Number | Distance from Airgun Array to Center of Cage (m) | Peak SPL (maximum) (dB re 1 µPa)* | Peak- SPL (dB re 1 µPa) (Peak-) | SEL (dB re 1 $\mu Pa^2 \cdot s$) | rms SPL (dB re 1 µPa) |
|---|---|---|---|---|---|
| 1 | 0** | 231 | 224 | 205 | 225 |
| 2 | 6.25 | 222 | 221 | 199 | 215 |
| 3 | 14.75 | 215 | 212 | 193 | 206 |
| 4 | 21 | 214 | 210 | 192 | 205 |
| 5 | 33.75 | 206 | 205 | 187 | 199 |
| Control*** | 160 south | 139 ± 7.7 | 138 ± 7.7 | 125 ± 4.0 | 105 ± 4.3 |

* Number of samples at cage locations 1 to 5 = 64; number of samples at Control = 13. Standard deviation provided for Control since this was more than 1 dB. This was not provided for other values since it was always less than 1 dB.
** Cage 1 was just above or below the airgun array, depending upon species.
*** Sound levels at the control cage represent ambient noise levels in the lake.
Peak SPL = peak sound pressure level; Peak- SPL = peak negative sound pressure level.

A comprehensive set of sound exposure data was obtained using a combination of real-time and autonomous recording systems to measure sounds at the airgun barge and at the cages before and during the complete study. This was necessary so that the effects on the fish (e.g., immediate or delayed mortality) could be correlated with the dose (sound) received by the fish.

The sounds from each shot were monitored (via hydrophone) and the results reviewed immediately following the shot to ensure that each was an acceptable replicate. In addition, the sound produced by every shot was recorded digitally so that information about each shot would be available, if needed, for later analysis. Average sound levels at each location are presented in **Table 3**. It should be noted that there was, as expected, slight variation in sound level from shot to shot, that was <± 1 dB. The metrics computed for each shot along with summary statistics including means and standard deviations can be found in **Appendix C**.

### 2.7.1 Acoustic Recorders

Two real-time systems, each consisting of an acoustic data acquisition and monitoring system (ADAMS; JASCO Applied Sciences) with a hydrophone and a laptop computer, were used to display and record acoustic data. Real-time analysis provided quality control feedback ensuring consistency of shot levels and fish exposure (**Appendix C** for details). As configured, the system was capable of recording high sound levels making it suitable for use near the airgun array. The real time systems were used to sample the acoustic field during the preliminary acoustic mapping and to monitor sound levels at the center of the two cages closest to the sound source when exposing fish to airgun sounds. Four autonomous multichannel acoustic recorders, mini version (AMAR Mini; JASCO Applied Sciences) were used to acquire the sound measurements used to determine the preliminary acoustic mapping and to monitor sound levels at the three cages farthest from the sound source and at a control location. The autonomous recorders were attached to moorings and deployed at the locations of the cages.

## 2.8 NECROPSY

Fish returned to the hatchery were monitored every 12 h for 7 days post-exposure. They were then euthanized, refrigerated for an average of about 15 h, and necropsied.

### 2.8.1 Necropsy Training

Necropsy (autopsy for animals) was performed by a team of investigators who were trained over several days (details of training and necropsy are provided in **Appendix D**). Initially, the necropsy team was shown images of potential damage effects and then trained in basic necropsy procedures to be used in this Study. This was followed by practice necropsy on the test specimens. At the end of training all of the investigators were competent in doing all phases of the necropsy procedure, including euthanization of fish, dissections, examination of tissue, and recording of data.

### 2.8.2 Necropsy Procedures

A detailed protocol for necropsy is provided in **Appendix D**. The start of the procedure had fish deeply anesthetized to a point of termination of respiration. This was done by placing the fish in a cooler that contained buffered anesthetic in aerated hatchery water at 13.9 $^{o}$C. The anesthetic was 750 ppm of tricaine methanesulfonate (MS-222[6]), a veterinary-approved anesthetic for fish to prevent potential pain to the fish. Fish were considered to have been euthanized once they showed no opercular movements for 10 min.

Following euthanasia, each fish was dip netted from the solution, had excess water removed by patting with a paper towel, wrapped in paper, labeled with time and day of refrigeration, and placed on a shelf in a walk-in cooler at 3.3 $^{o}$C. After of about 15 h of refrigeration the fish were removed from the cooler for necropsy. Refrigeration allowed for much more controlled necropsy procedures by allowing investigators to euthanize in the evening and then dissect the following day. This substantially cut down on the amount of time required of investigators in any one day.

[6] See **Appendix C** for discussion of MS-222 and fish carcass disposal. Source: Western Chemical Inc., Ferndale, WA http://www.wchemical.com/TRICAINE-S-MS-222-P43C7.aspx

The exposure of any individual fish was never revealed to investigators conducting the necropsies. Once a fish was removed from the chiller, the investigators carefully removed the paper and noted the date and time of euthanasia as well as the length of time in refrigeration. The mass, length, and tag number of the fish was noted and recorded. The fish was then placed into a dissecting tray and opened using surgical scissors starting at the vent (cloaca) and cutting anteriorly, ending at the pericardium (**Figures 15** and **17**). Great care was taken to ensure that the excision was medial and superficial and that organs of the peritoneum were not injured during the cutting process.

Fish were immediately evaluated to assess bruising, hemorrhaging, and swim bladder condition. After the internal organs and body wall were evaluated, these organs were carefully removed or shifted to complete a more thorough examination of the swim bladder. Digital photographs were taken of all tissue as it was dissected and the internal condition of tissues of interest was recorded.

After evaluation of the swim bladder, kidney condition was determined. The quantity of fat around the internal tissues was quite high in pallid sturgeon and so care was taken to not disturb the renal cavity and interconnecting vascularity while removing the fat. Removal of the fat allowed visualization of kidney and swim bladder.

Visualization of the swim bladder in paddlefish also required removal of a layer of fat. This also allowed for visualization of the entire kidney.

It was simpler to evaluate the swim bladder of the walleye than the other fish because the walleye's swim bladder was larger and ran the entire length of the body. Once the body cavity was opened, all internal organs could be detached and shed with one cut made to their posterior attachment to evaluate the state of the swim bladder. The size of the swim bladder made it more complicated to examine the renal cavity. To view the renal cavity, a medial incision was made in the swim bladder to deflate it and help expose the kidney.

Notations were made on a data sheet (**Appendix E**) about the condition of all tissues as they were evaluated. Following the complete necropsy, any final notes were made on the data sheet and the fish body and any removed tissues were disposed of. **Appendix F** describes disposal of fish tissues.

## 2.9 DATA MANAGEMENT

Data management protocols were established prior to initiating the Study and followed throughout to ensure data quality (**Appendix D**). The data management protocol included establishing databases and data sheets prior to initiating the Study; conducting quality control checks on all data recorded and entered into the databases; and maintaining and storing hardcopy, electronic, and metadata in multiple locations, including off-site.

Prior to initiating the Study, two databases (spreadsheets) were established in Microsoft Excel, one to store the fish tagging information and the second to store the fish exposure information and the results of the necropsies. Each database also contained a metadata spreadsheet. Necropsy data sheets were created based off the database to ensure the ease of data entry. Exposure data sheets for each species, which contained the randomized assignments of treatments, were created prior to going into the field. Refer to **Appendix E** for examples of data sheets.

Following data collection, the hardcopy data sheets and metadata notes were reviewed to ensure quality of the data, copied, and scanned for digital storage. The originals and copies of the data sheets were stored at two different locations. Data sheets also were saved in digital format on CSA servers. Scanned copies of all notes and data sheets also were backed up in two separate locations. Photographs of necropsied fish were downloaded daily and renamed with the species and tag number.

Data were entered into the database by one person and quality checked by a different investigator. All data entered into the database were quality checked before analysis. Additionally, a fatal injury code was assigned to each fish that had been necropsied. If a fish had a swim bladder rupture, kidney rupture, or kidney hemorrhage, the fish was recorded as having a mortal injury.

## 2.10 STATISTICAL ANALYSIS

A complete discussion of the statistical analysis is presented in **Appendix G**. The experimental units in the Study were individual cages with multiple fish inside. Each cage represented a binomial sample of $n_i$ fish, of which $x_i$ died or had mortal injury. There were five sound level classes (represented by Cages 1 to 5), with the sound level decreasing with distance from the sound source (**Table 3**). Each cage of fish received the sound generated by a single shot of the seismic array so that each cage of fish had separate measure of sound exposure. Two sound covariates were used as independent variables to assess the relationship between sound level (exposure) and death/mortal injury (response): peak negative sound pressure level (Peak- SPL) and sound exposure level (SEL). There also were controls where fish received the same handling as treatment fish except for exposure to sound. Because observations of death/mortal injury among control fish were made, an Abbott's adjustment (Finney, 1971) to the treatment fish was necessary.

The Abbott's adjustment (Finney, 1971) is based on the assumption that surviving handling (i.e., control survival) is independent of surviving the treatment, such that:

$$E\left(S_i\right) = S_C S_{T_i},$$

where

$S_i$ = observed survival of test fish exposed to handling and treatment $i$
$S_C$ = probability of surviving handling (i.e., control survival)
$S_{T_i}$ = probability of survival for fish exposed to a treatment $i$

Data analysis was therefore based on numbers of test fish that were alive and healthy (i.e., $n_i - x_i$) using generalized linear models (McCullagh and Nelder, 1989) with a binomial error structure and log-link. Analysis of deviance (ANODEV) was used to test hypotheses based on acquired data.

The analysis tested several hypotheses concerning the relationship between airgun array sound exposure and fish mortality/mortal injury $(M)$. These hypotheses included:

1. $\mathrm{H_o}$: $M_c = M_T$ vs. $\mathrm{H_a}$: $M_C \neq M_T$ — Control mortality/injury same as pooled treatments

2. $\mathrm{H_o}: M_c = M_i \quad \forall i$ — Control mortality/injury same as each treatment
   vs.
   $\mathrm{H_a}: M_C \neq M_i \quad \forall i$

3. $\mathrm{H_o}: M_c \geq M_{T_i}$ — Control mortality/injury $\geq$ *i*th treatment
   vs.
   $\mathrm{H_a}: M_C < M_{T_i}$

4. $\mathrm{H_o}: M \neq f(\text{PEAK-})$ — Mortality/injury not a function of Peak-
   vs.
   $\mathrm{H_a}: M = f(\text{PEAK-})$

5. $\mathrm{H_o}: M_i \neq f(\text{SEL})$ — Mortality/injury not a function of SEL
   vs.
   $\mathrm{H_a}: M_i = f(\text{SEL})$

Contingency tables and data plots also were used to summarize the test results. Estimates of death/mortal injury pooled across cages $(M_{T_i})$ within a distance class were plotted against average exposure levels. The empirical estimates of death/mortal injury (i.e., $\hat{M}_{T_i}$) were corrected for the death/mortal injury rate observed across the pooled controls (i.e., $\hat{M}_C$) according to Abbott's formula where

$$S_{T_i} = S_C S_{T_i}$$

or

$$M_{T_i} = \frac{\hat{M}_{T_i} - \hat{M}_C}{1 - \hat{M}_C}, \tag{1}$$

where $M_T$ is the control adjusted mortality/injury probability. For the summaries, data were pooled across replicate cages because sample sizes in the individual cages were too small to convey trends in the mortality data.

## 3.1 OVERVIEW OF RESULTS

As described in **Section 2.0**, test fish were exposed to sound in cages located horizontal distances of 0, 6.25, 14.75, 21, or 33.75 m from the anchored airgun array so that the peak negative sound pressure level (Peak- SPL) at Cage 1 (closest to the array) was about 224 dB re 1 µPa and the sound in Cages 2 through 5 were at Peak- sound levels of approximately 221, 212, 210, and 205 dB re 1 µPa, respectively (**Table 3**). The experimental units were individual cages containing fish. Each cage of fish was deployed separately and exposed to a single shot from the seismic array. The cage for the control fish was about 160 m south of the airgun array, but no seismic airgun sound was presented when controls were in the water and so they were exposed only to ambient sounds (**Table 3**).

Results for response to seismic sound were obtained for pallid sturgeon and paddlefish. In summary, as discussed below, there is no statistical evidence to suggest any mortal effect on either species for up to 7 days post-exposure. In addition, data for both the large and YOY walleye proved to be unusable because both treatment and control groups of this species responded poorly to all stages of handling (e.g., **Figure 14**). Thus, no reliable statistical inference for the effects of exposure to seismic sound could be drawn from the data acquired for walleye.


Figure 14. Walleye showing substantial bruising and other effects that precluded use in necropsy. In this case, note the reddening of tissue in the posterior region.

## 3.2 NECROPSY RESULTS

### 3.2.1 General Description

Necropsy results from pallid sturgeon (**Figures 15** and **16**) and paddlefish (**Figures 17** and **18**) show that there were no clear effects from exposure to the airgun compared with controls. **Figures 15** and **17** show a ventral view of the opened abdominal cavity, whereas **Figures 16** and **18** show views of the swim bladder and kidney with the other tissues moved aside.

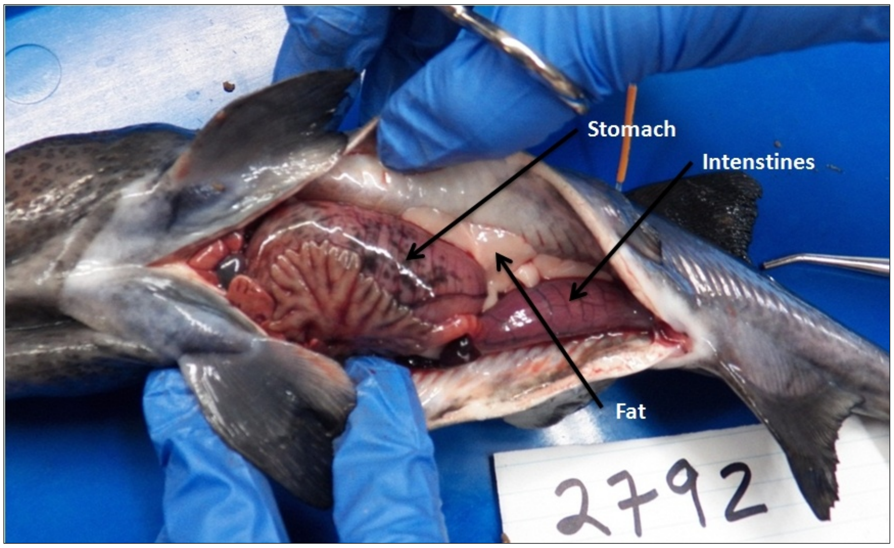



Figure 15. Internal anatomy of a necropsied paddlefish showing healthy structures. Head to the left. A ventral view of the fish with the abdominal cavity opened to show the viscera.

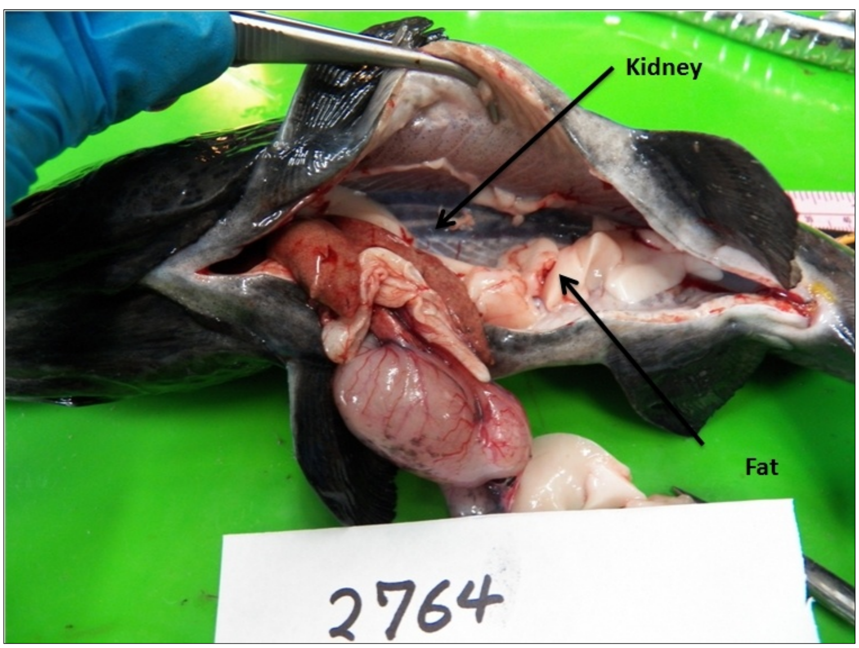



Figure 16. Paddlefish showing Internal anatomy. Most of the internal anatomy is reflected away to show the swim bladder.

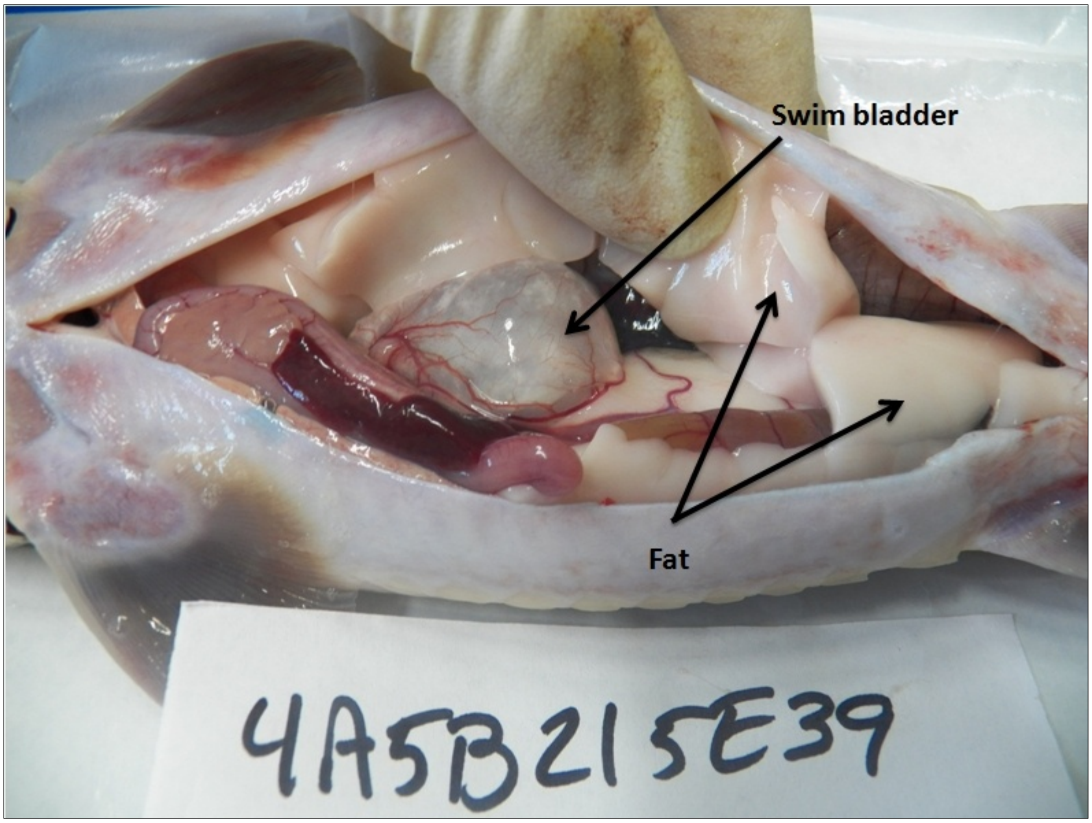



Figure 17. Pallid sturgeon showing internal anatomy.

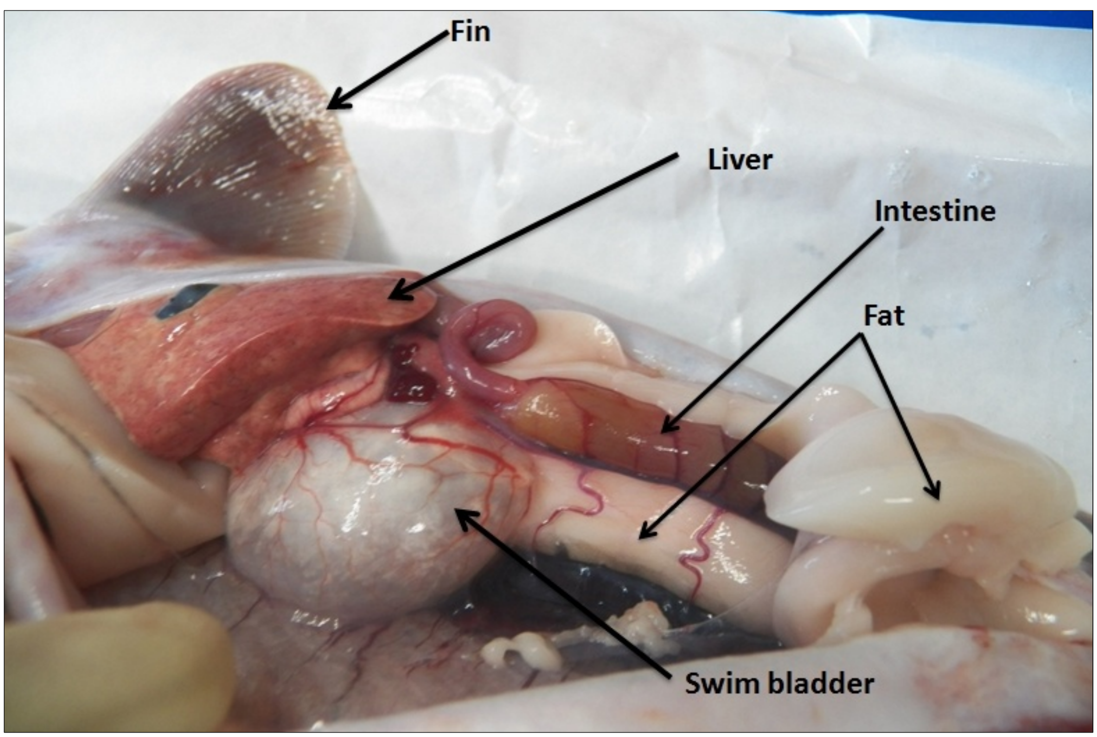



Figure 18. Pallid sturgeon showing healthy internal anatomy.

### 3.2.2 Statistical Results

A complete presentation of the statistical results as well as methodology is found in **Appendix C**.

#### *3.2.1.1 Pallid Sturgeon*

Three pallid sturgeon were placed in each cage prior to exposure. The resulting dataset consisted of five sets of exposures (exposure blocks) of samples from each of the five cages receiving sound and control cage not exposed to sound with the exception of 1 cage of fish in Block 4. The sound data for Cage 1 of Block 4 was compromised during acquisition and could not be recovered (**Table 4**).

Table 4. Raw counts of pallid sturgeon with and without mortal injury by test cage and associated levels of measured peak negative sound pressure (Peak- SPL) (±1 dB) and sound exposure level (SEL) used in data analysis. (Sound levels rounded. See **Appendix G**, attachment A, for sound levels to tenths of a dB).

| Exposure ID | Alive* | Injured** | Block | Treatment | Peak- SPL (dB re 1 μPa) | SEL (dB re 1 $\mu Pa^2 \cdot s$) |
|---|---|---|---|---|---|---|
| B1D1 | 2 | 1 | 1 | 1 | 224 | 205 |
| B1D2 | 3 | 0 | 1 | 2 | 222 | 199 |
| B1D3 | 3 | 0 | 1 | 3 | 211 | 191 |
| B1D4 | 2 | 1 | 1 | 4 | 210 | 190 |
| B1D5 | 3 | 0 | 1 | 5 | 206 | 186 |
| B1DC | 2 | 1 | 1 | Control | 0 | 0 |
| B2D1 | 3 | 0 | 2 | 1 | 225 | 206 |
| B2D2 | 2 | 1 | 2 | 2 | 222 | 200 |
| B2D3 | 2 | 1 | 2 | 3 | 212 | 192 |
| B2D4 | 2 | 1 | 2 | 4 | 211 | 191 |
| B2D5 | 3 | 0 | 2 | 5 | 206 | 187 |
| B2DC | 3 | 0 | 2 | Control | 0 | 0 |
| B3D1 | 3 | 0 | 3 | 1 | 225 | 206 |
| B3D2 | 1 | 1 | 3 | 2 | 223 | 200 |
| B3D3 | 3 | 0 | 3 | 3 | 214 | 193 |
| B3D4 | 3 | 0 | 3 | 4 | 211 | 192 |
| B3D5 | 3 | 0 | 3 | 5 | 205 | 186 |
| B3DC | 2 | 0 | 3 | Control | 0 | 0 |
| B4D2 | 2 | 1 | 4 | 2 | 222 | 200 |
| B4D3 | 2 | 1 | 4 | 3 | 213 | 192 |
| B4D4 | 2 | 1 | 4 | 4 | 211 | 191 |
| B4D5 | 1 | 2 | 4 | 5 | 206 | 186 |
| B4DC | 1 | 1 | 4 | Control | 0 | 0 |
| B5D1 | 3 | 0 | 5 | 1 | 225 | 206 |
| B5D2 | 3 | 0 | 5 | 2 | 223 | 200 |
| B5D3 | 3 | 0 | 5 | 3 | 212 | 192 |
| B5D4 | 3 | 0 | 5 | 4 | 210 | 191 |
| B5D5 | 3 | 0 | 5 | 5 | 206 | 186 |
| B5DC | 3 | 0 | 5 | Control | 0 | 0 |

*Alive and without injury.
**Mortality or mortal injury.

No pallid sturgeon mortalities coincident with sound exposure occurred and none died during the 7-day holding period. At the end of the holding period, treatment fish (exposed to sound) and control fish (not exposed to sound) were euthanized and necropsied to determine the frequency of occurrence of mortal injury (see **Section 2.8**). Mortal injuries were found both in fish exposed to sound and in controls (**Table 4**). Consequently, the analysis of the experimental data required adjustment for control effects.

An R x C contingency table (**Table 5**) displays the raw counts for the five different treatment groups (cage distance 1 through 5) plus controls, after pooling across replicates. The observed proportions of fish with mortal injuries among the treatments ranged from 0.0833 to 0.2143. The control fish had an observed mortal injury proportion of 0.1538. Pooling across the five treatment groups, the observed proportion of mortal injuries was 0.1549, which was nearly identical to the control rate. The R x C contingency table found no difference in proportions of mortal injury among the six groups of fish $\left(P\left(\chi_5^2 \geq 1.1893\right) = 0.9461\right)$.

Table 5. Counts of observed mortal injury by treatment group (proportion in parentheses) for pallid sturgeon. Treatment Groups (cages) 1 through 5 are in order of increasing distance from sound source. Chi-square test of homogeneity was not significant $\left(P\left(\chi_5^2 \geq 1.1873\right) = 0.9461\right)$.

| Observation | Treatment Group (Cage Number) and Control | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Control |
| Alive and healthy | 11 | 11 | 13 | 12 | 13 | 11 |
| | (0.9167) | (0.7857) | (0.8667) | (0.8000) | (0.8667) | (0.8462) |
| Mortal injury | 1 | 3 | 2 | 3 | 2 | 2 |
| | (0.0833) | (0.2143) | (0.1333) | (0.2000) | (0.1333) | (0.1538) |

Analysis of deviance (ANODEV) found no difference in the rate of mortal injury between the control and treatments pooled $\left(P\left(F_{1,27} \geq 0.0001\right) = 0.9924\right)$ or individually $\left(P\left(F \geq 0.2047\right) = 0.9572\right)$ (**Table 6**). In addition, none of the five test groups had significantly higher rates of mortal injury than the controls $\left(P \geq 0.3554\right)$ (**Table 6**).

Table 6. Summary of results from the analysis of deviance of mortal injury data from the pallid sturgeon experiment with null hypotheses tested, test statistics, and associated *P*-values.

| Null hypotheses | Test Statistic | *P*-value |
|---|---|---|
| $H_o\colon M_C = \bar{M}_T$ | $F_{1,27} = 0.0001$ | 0.9924 |
| $H_o\colon M_C = M_{T_i}; i = 1,\ldots,5$ | $F_{5,27} = 0.2047$ | 0.9572 |
| $H_o\colon M_C \geq M_{T_1}$ | $Z = -0.4991$ | 0.6912 |
| $H_o\colon M_C \geq M_{T_2}$ | $Z = 0.3709$ | 0.3554 |
| $H_o\colon M_C \geq M_{T_3}$ | $Z = -0.1408$ | 0.5560 |
| $H_o\colon M_C \geq M_{T_4}$ | $Z = 0.2934$ | 0.3846 |
| $H_o\colon M_C \geq M_{T_5}$ | $Z = -0.1408$ | 0.5560 |
| $H_o\colon M_{T_1} \neq f\left(\text{PEAK\_}\right)$ | $F_{1,27} = 0.0000$ | 0.9987 |
| $H_o\colon M_{T_1} \neq f\left(\text{SEL}\right)$ | $F_{1,27} = 0.0001$ | 0.9914 |

ANODEV also was used to test whether there was a significant relationship between the level of sound exposure and the rate of mortal injury. No relationship was found between the peak negative sound pressure level (Peak- SPL) and the rate of mortal injury ($P$ = 0.9987), nor between sound exposure level (SEL) and the rate of mortal injury ($P$ = 0.9914) (**Table 6**). Plots of the observed rates of mortal injury after correcting for controls illustrate no pattern for either Peak- or SEL (**Figure 19**).

a) Mortal injury vs. Peak- SPL

b) Mortal injury vs. SEL

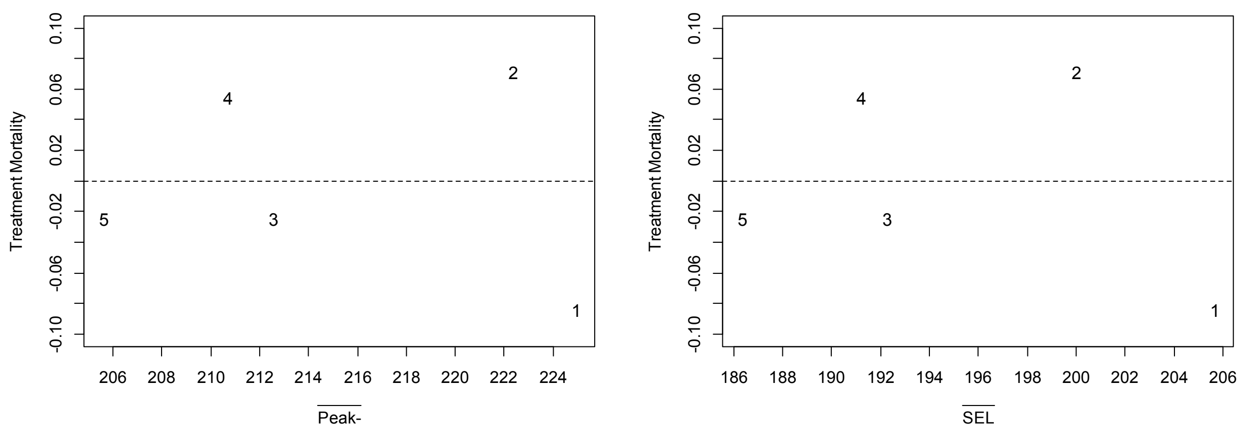



Figure 19. Scatterplots of observed rates of mortal injury after corrections for control rates against a) peak negative sound pressure level (Peak- SPL) and b) sound exposure level (SEL). Data were pooled over replicates and exposure levels were averaged. Treatments 1 to 5 are in order of increasing distance from sound source. (Peak- in units of dB re 1 µPa; SEL in units of dB re 1 µPa$^2$·s.)

Results of the analyses suggest at the SELs tested, there was no effect on mortality or mortal injury to pallid sturgeon from exposure to the impulsive sound generated by the airgun array.

### *3.2.1.2 Paddlefish*

For paddlefish the experimental details were the same except the study consisted of three blocks each, in turn, consisting of five treatment samples and one control sample. Each cage of paddlefish had four fish. Data for each cage are presented in **Table 7**.

An R x C contingency table (**Table 8**) displays the raw counts for the five different treatment groups plus controls after pooling across replicates. The observed proportions of fish with mortal injuries (no mortalities observed) among the treatments ranged from 0.0 to 0.3636 in a non-monotonic pattern. The overall proportion of mortal injuries among treatment fish was 0.16. The control fish had an observed proportion of 0.10 with mortal injury. The R x C contingency table found no differences in proportions with mortal injury among the six groups of fish $\left(P\left(\chi_5^2 \geq 6.5062\right) = 0.2600\right)$.

ANODEV found no difference in the rate of mortal injury between the controls and all treatments pooled $\left(P\left(F_{1,16} \geq 0.1775\right) = 0.6791\right)$ or individually $\left(P\left(F_{5,16} \geq 1.0829\right) = 0.4176\right)$ (**Table 8**). In addition, none of the five test groups had significantly higher rates of mortal injury than the controls ($P \geq$ 0.1167) (**Table 9**). The only treatment group that approached showing significantly higher mortal injury than the control group was the group in Cage 1, treatment 1 ($P$ = 0.1167) with an observed mortal injury proportion of 0.3636.

Table 7. Raw counts of paddlefish with and without mortal injury by test cage and associated levels of measured peak negative sound pressure (Peak- SPL) and sound exposure level (SEL) used in data analysis. (Sound levels rounded. See **Appendix G**, Attachment B for sound levels to tenths of a dB.)

| Exposure ID | Alive* | Injured** | Block | Treatment | Peak- SPL (dB re 1 μPa) | SEL (dB re 1 μPa$^2$·s) |
|---|---|---|---|---|---|---|
| B1D1 | 1 | 3 | 1 | 1 | 222 | 204 |
| B1D2 | 4 | 0 | 1 | 2 | 215 | 194 |
| B1D3 | 3 | 0 | 1 | 3 | 212 | 192 |
| B1D4 | 3 | 0 | 1 | 4 | 210 | 192 |
| B1D5 | 2 | 0 | 1 | 5 | 206 | 187 |
| B1DC | 3 | 0 | 1 | Control | 0 | 0 |
| B2D1 | 3 | 0 | 2 | 1 | 224 | 206 |
| B2D2 | 3 | 0 | 2 | 2 | 214 | 195 |
| B2D3 | 2 | 1 | 2 | 3 | 213 | 193 |
| B2D4 | 3 | 0 | 2 | 4 | 210 | 192 |
| B2D5 | 4 | 0 | 2 | 5 | 206 | 187 |
| B2DC | 3 | 0 | 2 | Control | 0 | 0 |
| B3D1 | 3 | 1 | 3 | 1 | 224 | 206 |
| B3D2 | 4 | 0 | 3 | 2 | 215 | 194 |
| B3D3 | 2 | 0 | 3 | 3 | 212 | 192 |
| B3D4 | 2 | 2 | 3 | 4 | 212 | 193 |
| B3D5 | 3 | 1 | 3 | 5 | 206 | 187 |
| B3DC | 3 | 1 | 3 | Control | 0 | 0 |

*Alive and without injury.
**Mortality or mortal injury.

Table 8. Counts of observed mortal injury by treatment group (proportion in parentheses) for paddlefish. Treatment groups (cages) 1 through 5 are in order of increasing distance from sound source. Chi-square test of homogeneity was not significant $\left(P\left(\chi_5^2 \geq 6.5062\right) = 0.2600\right)$.

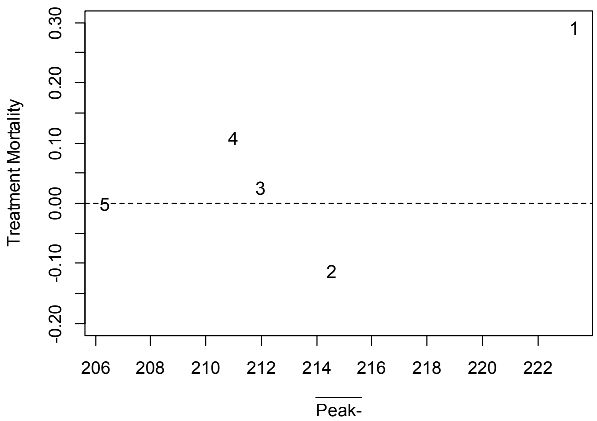
| Observation | Treatment Group (Cage Number) and Control | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Control |
| Alive and healthy | 7 | 11 | 7 | 8 | 9 | 9 |
| | (0.6364) | (1.0000) | (0.8750) | (0.8000) | (0.9000) | (0.9000) |
| Mortal injury | 4 | 0 | 1 | 2 | 1 | 1 |
| | (0.3636) | (0.0000) | (0.1250) | (0.2000) | (0.1000) | (0.1000) |

Table 9 Summary of results from the analysis of deviance of mortal injury data from the paddlefish experiment with null hypotheses tested, test statistics, and associated *P*-values.

| Null Hypotheses | Test Statistic | *P*-value |
|---|---|---|
| $\mathrm{H_o}\colon\ M_C = \bar{M}_T$ | $F_{1,16} = 0.1775$ | 0.6791 |
| $\mathrm{H_o}\colon\ M_C = M_{T_i};\ i = 1,\ldots,5$ | $F_{5,12} = 1.0829$ | 0.4176 |
| $\mathrm{H_o}\colon\ M_C \geq M_{T_1}$ | $Z = 1.1916$ | 0.1167 |
| $\mathrm{H_o}\colon\ M_C \geq M_{T_2}$ | $Z = -0.8629$ | 0.8059 |
| $\mathrm{H_o}\colon\ M_C \geq M_{T_3}$ | $Z = 0.1429$ | 0.4432 |
| $\mathrm{H_o}\colon\ M_C \geq M_{T_4}$ | $Z = 0.5351$ | 0.2963 |
| $\mathrm{H_o}\colon\ M_C \geq M_{T_5}$ | $Z = 0.0000$ | 0.5000 |
| $\mathrm{H_o}\colon\ M_{T_i} \neq f(\mathrm{PEAK\_})$ | $F_{1,16} = 0.2513$ | 0.6230 |
| $\mathrm{H_o}\colon\ M_{T_i} \neq f(\mathrm{SEL})$ | $F_{1,16} = 0.2743$ | 0.6077 |

The ANODEV was used also to test whether there was a relationship between the level of sound exposure and the rate of mortal injury. Neither peak negative sound pressure level (Peak- SPL) ($P$ = 0.6230) nor sound exposure level (SEL) ($P$ = 0.6077) was related to the rate of mortal injury. Tests of positive relationships would have $P$-values of 0.3115 and 0.3039, respectively. Plots of the observed rates of mortal injury corrected for controls illustrate no definitive pattern in mortal injury for either Peak- or SEL (**Figure 20**).

a) Mortal injury vs. Peak- SPL




b) Mortal injury vs. SEL

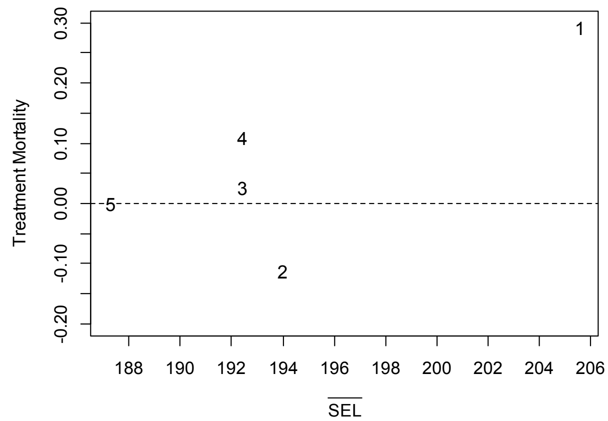



Figure 20. Scatterplots of observed rates of mortal injury after correction for control rates against a) peak negative sound pressure level (Peak- SPL) and b) sound exposure level (SEL). Data were pooled over replicates and exposure levels averaged. Treatments 1 to 5 are in order of increasing distance from sound source. (Peak- SPL in units of dB re 1 µPa; SEL in units of dB re 1 $\mu Pa^2 \cdot s$.)

Results of the analyses provide no definite evidence of increased mortality or mortal injury to paddlefish at the exposure level tests. There is marginal evidence ($P$ = 0.1167) that there might be elevated rates of mortal injury at the closest treatment level 1 with average Peak- of 223.3 dB re 1 µPa. However the small sample sizes make determining significant effects difficult at the individual treatment level.

### *3.2.1.3 Adult Walleye*

A series of handling challenges with adult walleye resulted in sample sizes too small for reliable statistical inference of the effects of sound exposure on these fish.

### *3.2.1.4 Young-of-Year Walleye*

No statistical inferences regarding the effects of sound exposure on small walleye were possible because there was 100% mortality associated with control fish.

# 4.0 Discussion

The single shot exposure paradigm used in this Study was selected because it was determined to be the best simulation of the proposed Stony Creek 3D seismic survey strategy. That plan calls for the seismic vessel carrying the airgun to move along preplanned transects where a single shot would be generated by the airgun array at each preplanned shot point. After a shot is completed the vessel would move on the order of 100 m to the next location where another shot would be fired. The distance traveled by the airgun vessel would most likely assure that if a fish were exposed to two shots, one shot would usually be much higher in energy than the other therefore any observed effect could be assumed to be a consequence primarily of the higher energy exposure. Thus, in the present experiment, it was decided that only a single shot would be appropriate to simulate the effective sound level to which fish would likely be exposed during the actual seismic survey.

## 4.1 OVERVIEW OF FINDINGS

This Study involved exposing three species of fish common to Lake Sakakawea and surrounding waters, pallid sturgeon, American paddlefish, and walleye, to sounds from the proposed airgun arrays that will be used in a seismic survey of the lake. However, results for walleye could not be used for reasons discussed in **Section 3.0**. The fish were in cages located at specific distances from the airgun (**Table 3**). The initial goal in the experimental design was to develop a dose-response function whereby the levels of sound received by fishes at different distances from the source could be quantitatively related to the response of the fishes to the sound exposure, in terms of mortality during or within 7 days of exposure. However, the results of the Study did not provide data that could be used to derive a dose-response function because no statistically significant response of test fish to seismic sound was detected. Even at the highest sound levels, there was no mortality in fish suspended at the center of the airgun array where the greatest energy was observed.

The overall goal of this Study was to test fish under acoustic conditions that are similar to those that they would be exposed to if there were an actual seismic survey. Clearly, the results are contrary to the expectation that there would be mortality of fish exposed to the impulsive airgun sound, at least to sturgeon and paddlefish exposed at the highest sound levels (approximately 224 dB re 1 µPa Peak-; approximately 205 dB re 1 $\mu Pa^2 \cdot s$ SEL, **Table 3**). The evaluation of mortality and mortal injury occurred over 7 days post-exposure. At the time the Study was completed on day 7 the extent of swim bladder or kidney rupture or hemorrhaging did not differ statistically between exposed and control fish. Thus, it may be concluded that the sound levels from the seismic airgun used in this Study (or planned for actual seismic surveys) was not sufficiently intense, in terms of negative overpressure magnitude, to cause mortality or mortal injury that could be associated with sound exposure within 7 days of exposure in sturgeon and paddlefish.

It is possible that the airgun could be fired repeatedly during an actual survey, possibly as frequently as once every few minutes. Therefore an alternative exposure scenario would have been to use multiple airgun shots to simulate fish being exposed to multiple shots. However, even if a fish were exposed to multiple airgun shots, the likelihood is that the sequence of exposures for freely swimming fish during the seismic survey would be a single high level exposure followed by one or more exposures at much lower levels. The number of possible combinations of multiple exposures is very large when considering uncertainties about the distribution of fish, their normal movement pattens, and any possible response to sound. However because of the high rate of loss of sound energy (25log[r] transmission loss) with distance in shallow water, the total energy of exposure would almost certainly be dominated by the initial exposure regardless of fish distribution, movement patterns, or behavioral response to sound. This would especially be true for exposure scenarios where the initial exposure was to a stationary fish in the immediate vicinity of the airgun array and subsequent exposures after the airgun array had been moved to other shot positions. There is insufficient information on the behavior and distribution of pallid sturgeon and paddlefish in Lake Sakakawea, particularly in the presence of a seismic survey vessel, to design an exposure scenario that would improve the response prediction capability of the simple exposure model used for this Study.

## 4.2 POTENTIAL BEHAVIORAL EFFECTS

It is important to emphasize that this Study focused on potential mortal injury effects of seismic airguns. The fish studied were caged to ensure that they were exposed to known sound levels. However, their behavior in the cages was not a study objective, nor would such behaviors be in any way relevant to how wild animals would respond to exposure to seismic airguns (Hawkins and Popper, 2012).

## 4.3 STUDY CONSIDERATIONS

### 4.3.1 Extrapolation of Data to Fishes of Different Sizes

The length of all animals of each species used in the Study was within one standard deviation of one another. This was done in order to eliminate size as a potential variable in the results. At the same time, there could be concern that the results cannot be extrapolated to larger or smaller fish. There are no available studies that have examined effects of impulsive sounds, including seismic airguns, on fish of different sizes. The one potentially relevant study that did examine effects of sounds from underwater explosions on fish of different sizes showed that as fish get larger it takes higher sound levels, on the order of 5 dB increase in SEL for each kilogram increase in fish mass, to show damage (Yelverton et al., 1975; also see analysis in Carlson et al., 2007 and in Popper and Hastings, 2009). Studies that observe the effect of exposure to intense sounds on fish over a range of sizes are needed to provide information necessary to characterize the expected differential in physiological response of fish of different sizes and age classes to impulsive sound. At the same time, these very limited results suggest that fish larger than those exposed in the Study would have even less likelihood of mortal injury than the fish exposed in the study.

### 4.3.2 Source of Fish

In the case of both pallid sturgeon and paddlefish, animals were spawned and raised in the hatchery. Adult walleye were wild animals, though YOY walleye were hatchery animals. It is possible that hatchery-raised animals could have a different hardiness or be physiologically “different” than wild animals, and thus wild pallid sturgeon and paddlefish might show physiological effects even though these were not seen in the hatchery animals. It is possible that body fat noted during necropsy could have been protective in hatchery animals by insulating tissues surrounding the swim bladder from its movements in the impulsive sound field. Such “insulation” would prevent the swim bladder walls from striking and damaging nearby tissues (Popper and Hastings, 2009; Halvorsen et al., 2011, 2012a; Stephenson et al., 2010; Carlson et al., 2012).

### 4.3.3 Acclimation to Depth

Fish use their swim bladder to manage their buoyancy at different depths. To do this, they add air to, or remove air from, the swim bladder as they change depth. Fish add air to the swim bladder either by gulping air at the surface of the water before they descend (physostomous species) or they use a special gland as part of the swim bladder to pump air from the blood into the chamber (physoclistous species) (see Stephenson et al., 2010). In either case, if the swim bladder is not properly inflated at the depth of the animal the fish cannot maintain its position in the water column, making it expend energy not otherwise required.

More importantly for this Study, if the swim bladder is not properly inflated the walls are not properly located with respect to surrounding tissues. As a consequence, when the animal is exposed to an impulsive source the walls do not move with the same amplitude or speed as they do in a fish with a normally inflated swim bladder. Thus, a fish that does not have proper swim bladder inflation for the depth at which it is exposed is less likely to show injuries than would a fish in which the swim bladder is properly inflated.

It is not clear whether the fish used in the Study were physiologically acclimated to the exposure depth or not. The fish were lowered to depth as soon as they were placed in the cages and then exposed to sound within about a minute of reaching depth. As a consequence, the physostomous sturgeon species (pallid sturgeon and paddlefish) may not have had sufficient time at the surface to gulp the air they would need to have a properly filled swim bladder at 2-m depth (approximately 120.9 kPa absolute pressure[7]) in the case of the paddlefish and 6 m (approximately 160.2 kPa absolute pressure) for the pallid sturgeon. Similarly, the physoclistous walleye were lowered to depth over a period of about one minute and then exposed to the sound within a minute. It is not known if the physoclistous fish would have been able to fill their swim bladders to achieve normal size before they were exposed to the sound.

## 4.4 NECROPSY RESULTS

Necropsy was done by a group of investigators who were trained prior to the Study to ensure uniform methods and results. The procedures adopted were very similar to those used and validated in a series of pile driving studies (e.g., Halvorsen et al., 2011, 2012a, 2012b; Casper et al., 2012).

Although the original intention of this Study was to examine all of the same tissues reported in the pile driving study, it was not possible due to the time available for this element of the study and the length of time necessary to thoroughly examine large fish. Initial experience with the adult sturgeon and walleye made it clear that on the order of 30 to 45 minutes per fish would be required to complete an assessment for a full panel of potential injuries. In order to remain within schedule and budget, the group of injuries assessed was reduced to only those judged to be mortal. Thus, the modified injury panel reduced necropsy to examining for injuries that were expected to cause mortality—injuries of the swim bladder and kidneys. However, even necropsy for the reduced group of injuries required very careful dissections to avoid causing damage to tissues and bleeding, which could complicate the necropsy and confound interpretation of observations.

[7] Atmospheric pressure at sea level is about 101.3 kPa.

There was no difference in the extent of swim bladder or kidney ruptures or hemorrhaging between exposed and control fish for pallid sturgeon and paddlefish. Therefore, no injuries could be attributed to sound exposure.

## 4.5 IMPLICATIONS OF RESULTS TO OTHER SEISMIC STUDIES

It is concluded that although each seismic survey differs in the size of the airgun array, operational water depths, and in the species potentially affected, the results from this Study suggest levels of impulsive seismic airgun sound to which adult fish can be exposed without immediate mortality. Thus, it is clear from the results of this study that pallid sturgeon and paddlefish with body mass on the order of 200 to 400 g exposed to a received single impulse sound exposure level (SEL) of 205 dB re 1 $\mu Pa^2 \cdot s$ did not die immediately or within 7 days of exposure, and that the probability of mortal injury did not differ between exposed and control fish.

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# APPENDICES

# APPENDIX A

## Species Referenced in Report – Common and Scientific Names

American paddlefish – see Paddlefish
Australian pink snapper - *Pagrus auratus*
Broad whitefish - *Coregonus nasus*
Common sole - *Solea solea*
Lake chub - *Couesius plumbeus*
Northern pike - *Esox lucius*
Paddlefish - *Polyodon spathula*
Pallid sturgeon – *Scaphirhynchus albus*
Rock fish - *Sebastes*
Walleye - *Sander vitreus*

# APPENDIX B

## Glossary

| | |
|---|---|
| 3D | Three dimensional (in reference to seismic study). |
| Acoustic intensity | The work done per unit area and per unit time by a sound wave on the medium as it propagates. The units of acoustic energy flux are Joules per square meter per second ($J/m^2$-s) or watts per square meter ($W/m^2$). The acoustic energy flux is also called the acoustic intensity. |
| dB (decibel) | A logarithmic scale most commonly for reporting levels of sound. The actual sound measurement is compared to a fixed reference level and the decibel value is defined as 10 $\log_{10,}$(actual/reference), where (actual/reference) is a power ratio. Because sound power is usually proportional to sound pressure squared, the decibel value for sound pressure is 20$\log_{10}$ (actual pressure/reference pressure). As noted above, the standard reference for underwater sound pressure is 1 micro-Pascal (µPa). The dB symbol is followed by a second symbol identifying the specific reference value (i.e., dB re 1 µPa). A difference of 20 dB corresponds to a factor of 10 in sound pressure. |
| Fish[8] | One or more representatives of a single species of fish. |
| Fishes | More than one species of fish. |
| Fork length | Length of a fish measured from the tip of the snout (nose) to the end of the caudal (tail) fin rays (bones). |
| Hz (Hertz) | The units of frequency where 1 Hertz = 1 cycle per second. The abbreviation for Hertz is Hz. |
| Impulse | See impulse sound. |
| Impulse or impulsive sound | Transient sound produced by a rapid release of energy, usually electrical or chemical such as circuit breakers or explosives. Impulse sound has very short duration and high peak sound pressure relative to a continuous sound of comparable mean level |
| Mortal injury | An injury that results in death. |
| Necropsy | An autopsy done on an animal. |
| Opercular movement | Movement of the covering over the gills in fish. The opercules move during respiration. Lack of movement of the opercules is indicative of death. |
| Peak amplitude | The maximum deviation between the sound pressure and the ambient hydrostatic pressure. Sometimes described and measured as half peak to peak. |
| Peak sound pressure | The highest pressure above or below ambient that is associated with a sound wave. |

[8] Note, usage for “fish” and “fishes” is common usage within the ichthyological community.

| | |
|---|---|
| Peak overpressures | Overpressure is the pressure above the ambient level that occurs in an impulse sound such as an explosion. The peak overpressure is the highest pressure above ambient. |
| Physoclists | See Physostomes. |
| Physostomes | Fish species in which the swim bladder is connected to the oesophagus by a thin tube. Air to fill the swim bladder is swallowed by the fish and is directed to the swim bladder. Air removal from the swim bladder is by expulsion through this tube to the esophagus. Physoclistous fishes have no such connection. Instead, they add gas to the swim bladder using a highly specialized gas secreting system called the rete mirabile, which lies in the wall of the swim bladder and extracts gas from the blood using a counter-current system, much like that found in the kidney, to remove wastes from the blood. Removal of gas from the swim bladder occurs by reabsorption into the blood. |
| Pulse | A transient sound wave having finite time duration. A pulse may consist of one too many sinusoidal cycles at a single frequency, or it may contain many frequencies and have an irregular waveform. |
| rms[9] | Measure of the average pressure or as the "effective" pressure over the duration of an acoustic event, such as the emission of one acoustic pulse. Because the window length, *T*, is a divisor, pulses more spread out in time have a lower rms SPL for the same total acoustic energy. |
| SEL | See Sound Exposure Level; Sound Exposure Level, Single Strike; Sound Exposure Level, Cumulative. |
| Sound exposure | The integral over all time of the square of the sound pressure of a transient waveform. |
| Sound exposure level (SEL)[1] | Time integral of the squared pressure in a stated frequency band over a stated time interval or event. |
| Sound exposure level, single strike ($SEL_{ss}$) | See SEL. $SEL_{ss}$ is the energy in a single impulsive signal. |
| Sound exposure level, cumulative ($SEL_{cum}$) | The total energy in all of the signals to which an animal is exposed. It is expressed as: $SEL_{cum} = SEL_{ss} + Log_{10}$(number of signals). |

[9] Definitions with this footnote number are from the JASCO report in **Appendix E**.

| | |
|---|---|
| Sound pressure level (SPL) | The sound pressure level or SPL is an expression of the sound pressure using the decibel (dB) scale and the standard reference pressures of 1 µPa for water and biological tissues, and 20 µPa for air and other gases. Sound pressure is the force per unit area exerted by a sound wave above and below the ambient or static equilibrium pressure is called the acoustic pressure or sound pressure. The units of pressure are pounds per square inch (psi) or, in the SI system of units, Pascals (Pa). In underwater acoustics the standard reference is one-millionth of a Pascal, called a micro-Pascal (1 µPa). The conventional definition of sound pressure level is in terms of root mean square pressure. |
| Source level | Characterizes the sound power radiated by an underwater sound source expressed in decibels. Often expressed as the SPL at a standard reference distance from a point monopole, placed in a lossless uniform medium and extending to infinity in all directions. |
| Swim bladder | A gas (generally air) filled chamber found in the abdominal cavity of many species of bony fish, but not in cartilaginous fishes. The swim bladder serves in buoyancy control. In many species the swim bladder may also serve as a radiating device for sound production and/or as a pressure receiving structure that enhances hearing bandwidth and sensitivity. |

# APPENDIX C

## JASCO Report on Sounds

# Acoustic Measurements of Seismic Airgun Sounds in Lake Sakakawea for Determining Effects on Pallid Sturgeon and Other Key Fish Species

*Submitted to:*
John Young
Continental Shelf Associates, Inc.
*CSA Job Number 2480*

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November 2012

P001187

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# Contents

# Figures

# Tables

# 1. Methods: Acoustic Monitoring

The experiments were conducted in a narrow channel (150–200 m wide) with steep sides and a flat bottom (about 10.5 m deep) in Lake Sakakawea near Pick City, ND. Airguns were deployed from a small barge anchored in the center of the channel and caged fish were located along the center line of the channel leading away from the airgun barge. The team conducted preliminary acoustic mapping to determine cage placements for the experiment. Two types of acoustic recording systems were used: 1) real time monitoring systems (Acoustic Data Acquisition and Monitoring System or ADAMS) and 2) autonomous recorders (Autonomous Multichannel Acoustic Recorders or AMARs). For the preliminary acoustic mapping, an ADAMS was deployed from a pontoon boat at various locations and AMARs were placed at candidate cage locations (see **Section 1.3**). This initial mapping allowed cage locations to be chosen based on the level of expected acoustic exposure and provided a method to confirm equipment function and cross check recording levels.

During the actual experiment of exposing caged fish to airgun sounds, an ADAMS was used in the two cages closest to the airguns and AMARs were placed at the three farthest cages. An AMAR was also placed at the control cage (see **Section 1.4**).

## *1.1. Acoustic Recorders*

Two real-time systems, each consisting of an Acoustic Data Acquisition and Monitoring System (ADAMS; JASCO Applied Sciences) with a TC4034 hydrophone (RESON) powered by an EC6067 charge amplifier (RESON) and a laptop computer, were used to display and record acoustic data (**Figure 1**). With the TC4034 hydrophone, this system can record sound levels up to 237 dB re 1 μPa and is suitable for use near the airgun array. A sample rate of 64 000 samples per second was used with files of 10 min recording duration. The real-time systems were used to sample the acoustic field during the preliminary acoustic mapping and to monitor sound levels in the two cages closest to the sound source during the exposure experiment. To monitor sound levels during acoustic exposure, the hydrophones were placed at the center of the cages and 60 m cables were run to shore to connect to the ADAMS.

Four Autonomous Multichannel Acoustic Recorders (AMARs; **Figure 2**) were used to determine the preliminary acoustic mapping and to monitor sound levels at the three cages farthest from the sound source and at the control location. Low-sensitivity (–211 dB re 1 V/Pa) M8K hydrophones (GeoSpectrum Technologies) were used to give an effective recording range up to 218 dB peak sound level. A higher-sensitivity (–199 dB re 1 V/Pa) M8H hydrophones (GeoSpectrum Technologies) was used at the control location. The sampling rate was 64 000 samples per second at 24-bit resolution. These autonomous recorders were attached to moorings (see **Section 1.2**) and deployed at the locations of the cages. Data was acquired continuously while the recorders were deployed.

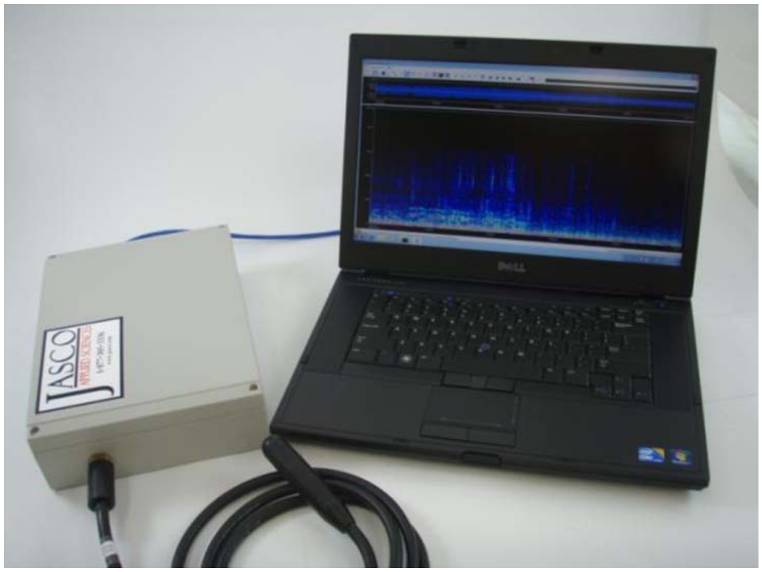

Figure 1. The JASCO Acoustic Data Acquisition and Monitoring System (ADAMS), shown with one hydrophone and a laptop, used for real-time monitoring and data acquisition.

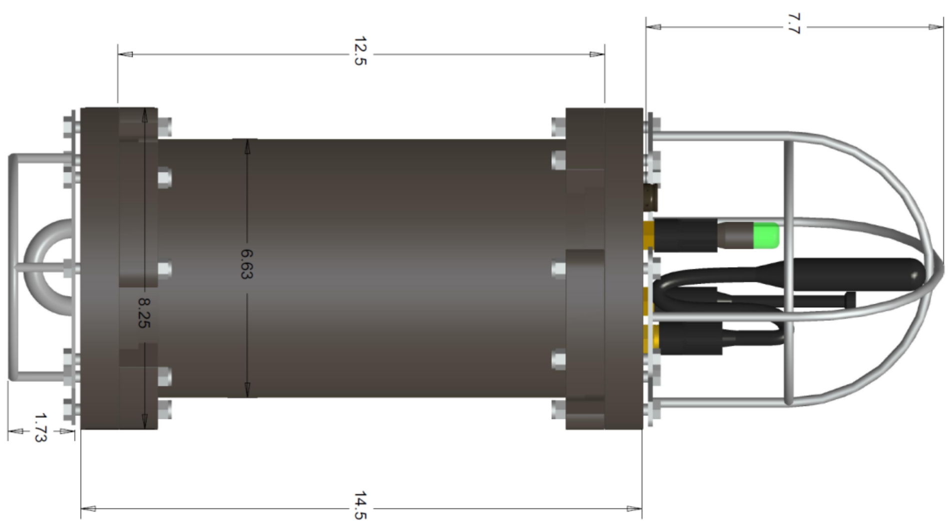


Figure 2. The JASCO Autonomous Multichannel Acoustic Recorder (AMAR). Dimensions are in inches.

## 1.2. Moorings

Each AMAR mooring consisted of an aluminum plate (on which the recorder was mounted), lifting brackets, a subsurface float, a ground line, and a surface float (**Figure 3**). With the AMAR attached to the aluminum mooring plate, the hydrophone was attached to the subsurface-float line so that the hydrophone would float 3 m off the bottom. The lifting brackets were used to lower the mooring to the bottom, and the surface float and ground line were used for retrieval.

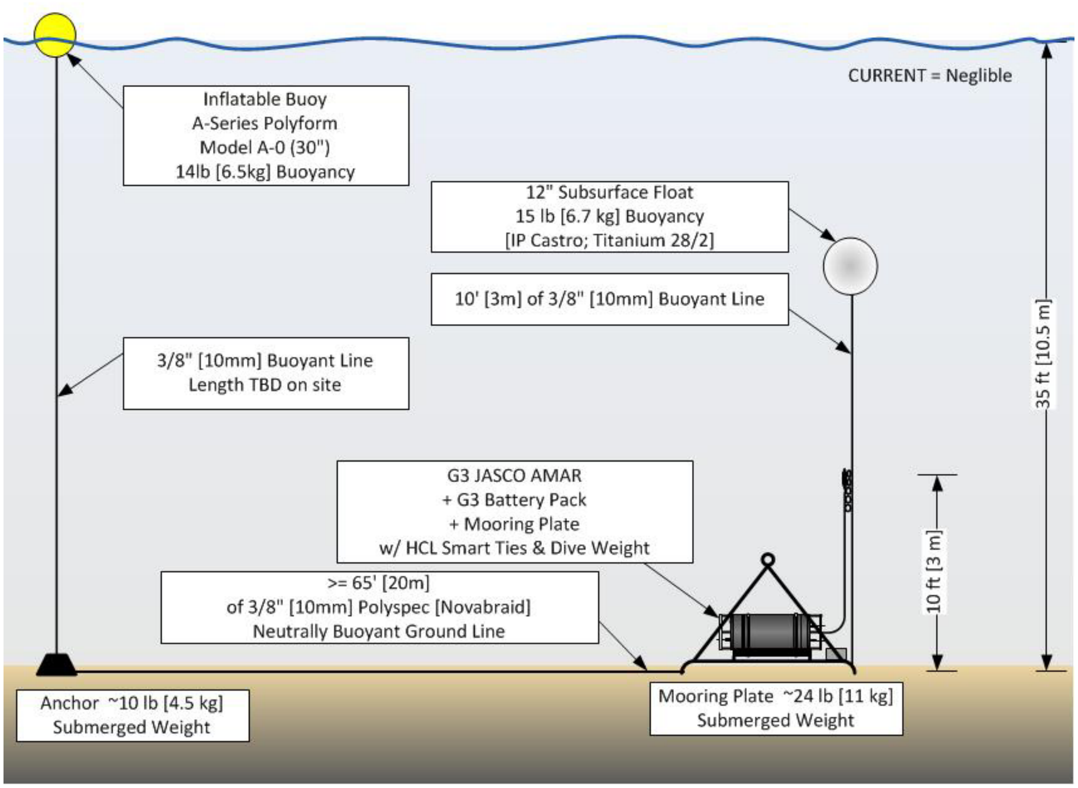


Figure 3. Mooring design for the Autonomous Multichannel Acoustic Recorders (AMARs).

## 1.3. Preliminary Measurements for Cage Placement

Initial planning called for each fish exposure cage to be at a sound level 6 dB lower than the next closest cage to the source; therefore, preliminary acoustic mapping of the sound field was needed to determine at what ranges to place the cages. Preliminary sound field measurements were obtained during setup and testing of the airgun array using the real-time ADAMS system at approximately 16, 32, 64, and 128 m from the array at three depths (2 and 5 m below the surface and 1 m above the bottom). Sampling with the real-time system was performed from a pontoon boat with the 16 and 32 m locations measured with a Leica Disto D5 digital handheld laser range finder (laser measurement accuracy 1 mm) and the 64 and 128 m locations with Garmin 78sc handheld GPS unit (accuracy ±3 m). AMARs were also used to sample the sound field. The AMARS were placed 10, 13.8, 19.2, 36.3, and 68 m relative to the barge using GPS for locations (the accuracy was ±3 m).

**Figure 4** shows the peak sound pressure level (SPL) as a function of slant range (i.e., range to center of the array accounting for the depths of the source and the receiver) for the ADAMS (open symbols) and AMARs (closed symbols). The ADAMS data are shown for the three depths at four distances and two shots at each position/depth combination; the AMAR data are means of 24 shots. The sound levels recorded by the AMARs were consistent; the standard deviation was small (typically 0.5 dB) so no error bars are shown.

The logarithmic function $245.36-25.03\log_{10}(r)$, where $r$ is the range, fit the data well ($R^2 = 0.9513$). The logarithmic function was used as a transmission loss curve to determine the distances at which to place the fish exposure cages. The cage locations were chosen to maximize exposure level. Cage 1 was suspended directly under the airgun array for a slant range of 1 m for paddlefish and 3 m for pallid sturgeon and walleye, and an estimated peak SPL above 226 dB re 1 µPa. The other cages were suspended from a line outstretched from the front of the barge (see **Section** 1.4) at slant ranges of approximately 10, 16.5, 22, and 35.5 m for Cages 2 through 5, respectively. The estimated zero-to-peak exposure levels for Cages 2 through 5 were 224, 216, 212, and 206 dB re 1 µPa, respectively. The recording limit for the AMARs (Cages 3, 4, and 5) was a peak level of 218 dB re 1 µPa, so Cage 3 was placed at a location expected to receive 216 dB instead of 218 dB to ensure the hydrophone would not saturate.

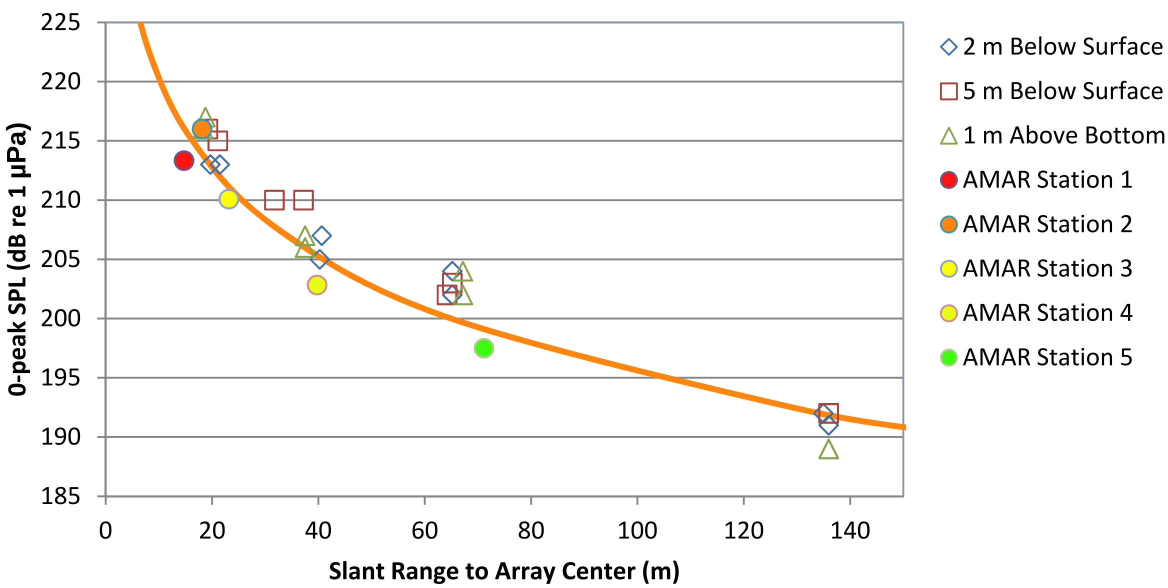


Figure 4. Peak sound pressure level (SPL) with slant range from the seismic airgun array used for determining the ranges at which to place the fish exposure cages. The equation of the curve fitting line is $245 - 25\log_{10}(r)$, where $r$ is the slant range from the source and $R^2$ = 0.9513.

## 1.4. Experimental Layout

To place the cages and autonomous recorders, a floating line was stretched from the front of the barge to a float anchored about 100 m down the channel from the barge. Floats were attached to the floating line at 3, 13, 18, and 32 m and used to suspend the cages and mark the AMAR deployment locations (**Figure 5**, **Figure 6**). The ranges from the barge to the floats indicating cage locations were measured with the Leica Disto D5 laser range finder: 6.25, 14.75, 21, and 33.75 m horizontal distance from the airgun array center. The cage locations, including the control cage, at the test site are shown in **Figure 7**.

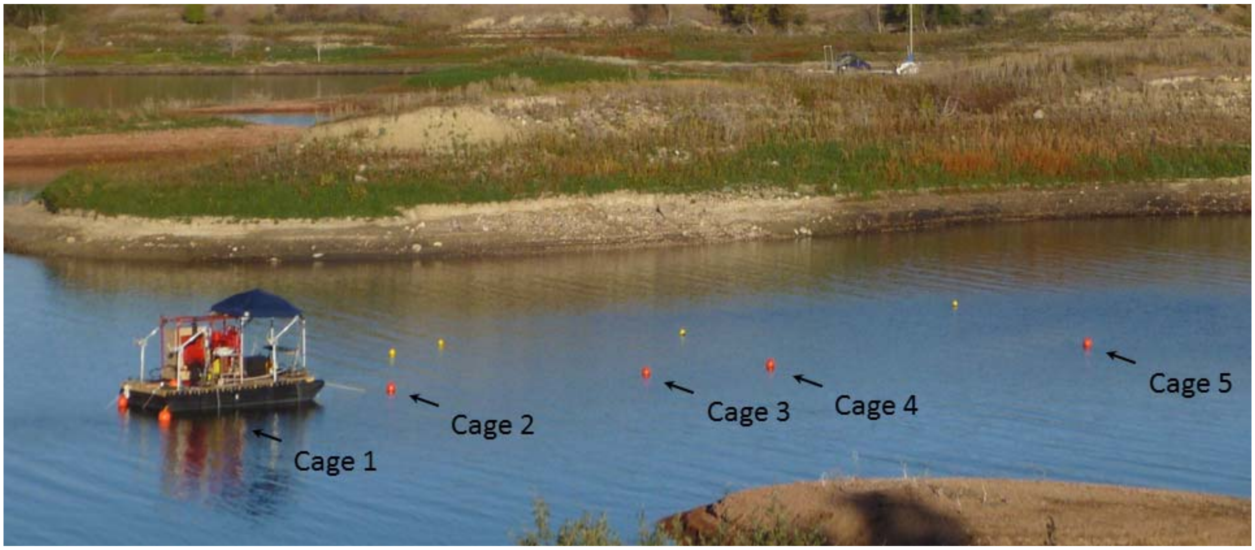


Figure 5. Airgun barge and fish exposure cage locations in the test area of Lake Sakakawea, ND. Red floats indicate cage and Autonomous Multichannel Acoustic Recorder (AMAR) locations. Yellow floats are surface floats used for AMAR retrieval (they do not indicate the location of the AMARs). Airguns were hung from davits near the corners of the barge.

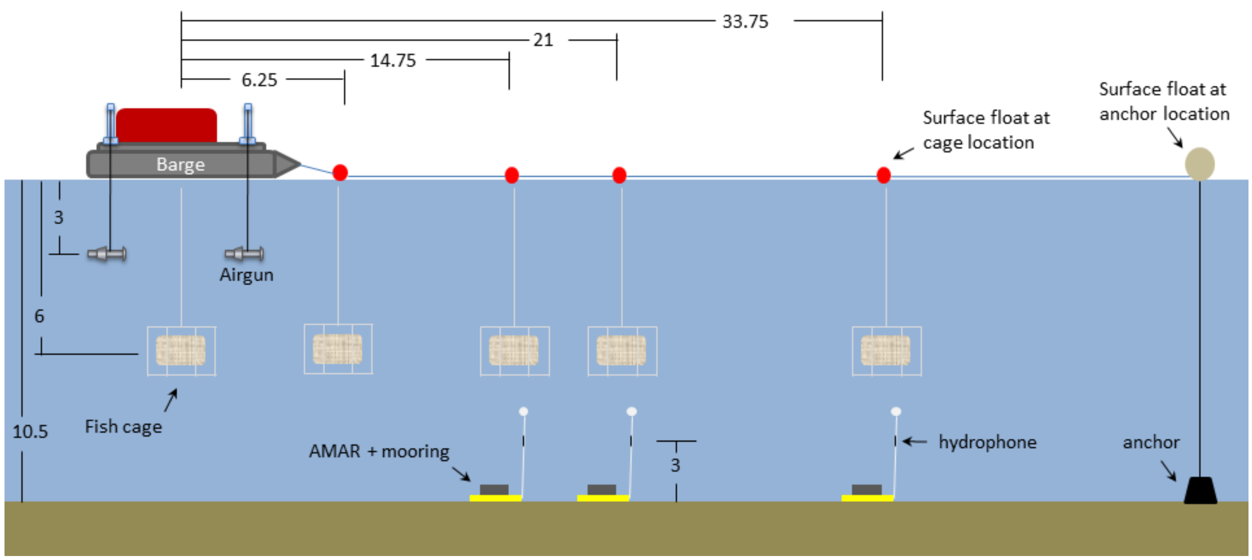


Figure 6. Schematic diagram of experimental setup showing locations of fish cages and autonomous recorders relative to the center of the airgun array. Dimensions are in meters.


Figure 7. Locations of the fish cages and acoustic recorders within the test area of Lake Sakakawea.

## 1.5. Data Analysis

Seismic events were detected automatically with a threshold detector in SpectroPlotter (JASCO Applied Sciences). The airgun shots were much louder than all other sounds (such as maneuvering boats) and were detected easily. The exposure level of fish at the control location was found by manually selecting the time period when the fish were in the control cage. Analysis of the seismic events and the manually-selected time periods included, root-mean-square (rms) SPL, sound exposure level (SEL), and zero-to-peak, zero-to-positive-going peak, zero to negative-going peak, and peak-to-peak levels. In addition, analysis of the seismic events included the time of the shot and duration

### 1.5.1. Acoustic Metrics

Underwater sound amplitude is measured in decibels (dB) relative to a fixed reference pressure of $p_o$ = 1 μPa; however, the loudness of impulsive noise, e.g., from seismic airguns, is not, in general, proportional to instantaneous acoustic pressure and so several sound level metrics are commonly used to evaluate the loudness of impulsive noise and its effects on marine life.

The zero-to-peak SPL, or peak SPL ($L_{pk}$, dB re 1 μPa), is the maximum instantaneous sound pressure level (either compression or rarefaction) in a stated frequency band attained by an impulse, $p(t)$:

$$L_{\mathrm{pk}} = 10\log_{10}\left(\frac{\max\left(\left|p^2(t)\right|\right)}{p_{\mathrm{o}}^2}\right) \tag{1}$$

Barotrauma may be more associated with the rarefactive, or negative, pressure of the acoustic wave (Carlson 2012). For that reason the zero-to-peak− (the maximum instantaneous rarefaction of the impulse), and the zero-to-peak+ (the maximum instantaneous compression), were also determined.

The root-mean square (rms) SPL ($L_p$, dB re 1 µPa) is the rms pressure level in a stated frequency band over a time window ($T$, s) containing the pulse:

$$L_p = 10\log_{10}\left(\frac{1}{T}\frac{\int_T p^2(t)dt}{p_{\mathrm{o}}^2}\right) \tag{2}$$

The rms SPL can be thought as a measure of the average pressure or as the "effective" pressure over the duration of an acoustic event, such as the emission of one acoustic pulse. Because the window length, $T$, is a divisor, pulses more spread out in time have a lower rms SPL for the same total acoustic energy.

The SEL ($L_E$, dB re 1 µPa$^2$·s) is the time integral of the squared pressure in a stated frequency band over a stated time interval or event. The per-pulse SEL is calculated over the time window containing the pulse:

$$L_E = 10\log_{10}\left(\int_{T_{100}} p^2(t)dt \Big/ T_{\mathrm{o}}p_{\mathrm{o}}^2\right) \tag{3}$$

where $T_{\mathrm{o}}$ is a reference time interval of 1 s. For practical purposes of defining the onset and end of a pulse, a 90% time window is defined that starts when the pulse attains 5% of its maximal squared pressure (or energy) and ends when the pulse reaches 95%. This 90% energy time window defines the duration of the pulse and is the time window over which integration is performed to calculate SEL. A correction of 0.5 dB is then added to the SEL value to account for the pulse energy outside of the 90% window. The per-pulse SELrepresents the total acoustic energy delivered over the duration of the acoustic event at a receiver location. It is a measure of sound energy (or exposure) rather than sound pressure, and can be a cumulative metric if it is calculated over time periods containing multiple pulses.

# 2. Results: Sound Levels at the Fish Cages

Experiments exposing fish to airgun sounds were conducted over three days, from 13 to 15 Sep 2012. The airgun shots were much louder than ambient sound levels and were easily distinguished from background (**Figure 8**), a simple threshold detector in the SpectroPlotter software was used to detect the occurrence of a shot. **Table 1** shows a statistical summary of the peak sound pressure levels at the cages and indicates that the shots were consistent, having a standard deviation less than 1.5 dB. Shot metrics for each species are summarized in **Tables 2–4**. Only one cage was loaded with fish for each airgun shot, and the cage to be loaded was selected randomly. The metrics in **Tables 2**, **3**, and **4** show the acoustic metrics at the fish-filled cage for each airgun shot. **Tables 5–7** show acoustic metrics at the control location when fish were in the control cage.

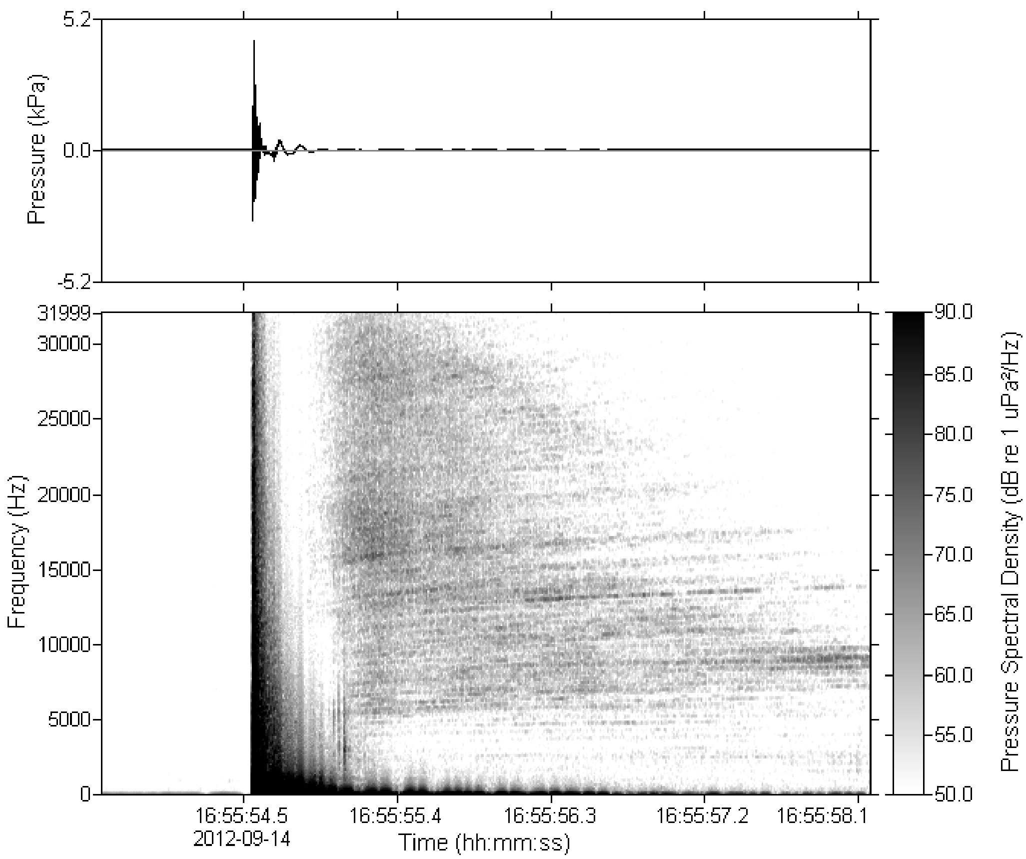


Figure 8. Time waveform and spectrogram of an airgun shot at Cage 3.

Table 1. Mean (± SD) peak sound pressure levels and durations of airgun shots at each cage for each day of the exposure experiment.

| Cage | 13 Sep 2012 (*n* = 15) | | 14 Sep 2012 (*n* = 24) | | 15 Sep 2012 (*n* = 25) | |
|---|---|---|---|---|---|---|
| | 0 to Peak SPL (dB re 1 µPa) | Duration (ms) | 0 to Peak SPL (dB re 1 µPa) | Duration (ms) | 0 to Peak SPL (dB re 1 µPa) | Duration (s) |
| 1 | 230.78 ± 1.43 | 8.5 ± 0.83 | 231.36 ± 0.46 | 10.3 ± 1.23 | 230.97 ± 0.36 | 10.6 ± 1.83 |
| 2 | 220.83 ± 0.65 | 19.5 ± 0.52 | 222.56 ± 0.41 | 22.2 ± 3.32 | 224.06 ± 0.85 | 23.0 ± 2.35 |
| 3 | 215.47 ± 0.43 | 36.7 ± 2.46 | 215.32 ± 0.31 | 39.0 ± 0.30 | 215.70 ± 0.59 | 36.2 ± 2.35 |
| 4 | 214.70 ± 0.57 | 37.6 ± 1.12 | 214.22 ± 0.41 | 38.8 ± 0.42 | 214.68 ± 0.50 | 37.9 ± 5.26 |
| 5 | 206.95 ± 1.18 | 45.1 ± 1.34 | 205.99 ± 0.65 | 45.9 ± 0.41 | 206.46 ± 0.86 | 45.0 ± 1.23 |

Table 2. Shot summary metrics for paddlefish on 13 Sep 2012.

| Time (UTC) | Cage | Duration (ms) | rms SPL (dB re 1 µPa) | SEL (dB re 1 µPa$^2$·s) | Peak SPL (dB re 1 µPa) | Peak+ SPL (dB re 1 µPa) | Peak– SPL (dB re 1 µPa) | Peak-peak SPL (dB re 1 µPa) |
|---|---|---|---|---|---|---|---|---|
| 21:44:58 | 5 | 46 | 199.36 | 186.96 | 206.41 | 205.60 | 206.41 | 212.03 |
| 22:01:17 | 1 | 10 | 223.80 | 204.90 | 232.65 | 232.65 | 222.26 | 234.95 |
| 22:18:16 | 2 | 20 | 210.02 | 193.91 | 221.34 | 221.34 | 214.70 | 224.66 |
| 22:23:38 | 4 | 37 | 205.56 | 192.11 | 214.88 | 214.88 | 210.29 | 218.91 |
| 22:31:54 | 3 | 39 | 205.43 | 192.23 | 215.23 | 215.23 | 211.53 | 219.60 |
| 22:37:30 | 4 | 37 | 205.88 | 192.44 | 215.27 | 215.27 | 210.21 | 219.12 |
| 22:49:21 | 2 | 20 | 209.85 | 193.68 | 220.01 | 220.01 | 214.25 | 223.62 |
| 22:56:19 | 5 | 45 | 199.99 | 187.40 | 207.59 | 207.59 | 206.23 | 212.96 |
| 22:01:09 | 3 | 36 | 206.52 | 193.00 | 215.88 | 215.88 | 212.71 | 220.46 |
| 23:14:49 | 1 | 10 | 224.74 | 205.77 | 233.35 | 233.35 | 223.73 | 235.83 |
| 23:34:02 | 1 | 10 | 224.89 | 206.00 | 233.46 | 233.46 | 223.94 | 235.97 |
| 23:43:40 | 3 | 39 | 205.18 | 192.00 | 215.14 | 215.14 | 211.63 | 219.58 |
| 23:47:42 | 5 | 43 | 200.03 | 187.40 | 207.87 | 207.87 | 206.30 | 213.14 |
| 23:52:30 | 2 | 19 | 210.54 | 194.32 | 219.81 | 219.81 | 214.57 | 223.60 |
| 23:56:33 | 4 | 36 | 206.22 | 192.64 | 215.46 | 215.46 | 212.42 | 220.09 |

Table 3. Shot summary metrics for pallid sturgeon on 14 Sep 2012.

| Time (UTC) | Cage | Duration (ms) | rms SPL (dB re 1 µPa) | SEL (dB re 1 µPa$^2$·s) | Peak SPL (dB re 1 µPa) | Peak+ SPL (dB re 1 µPa) | Peak− SPL (dB re 1 µPa) | Peak-Peak SPL (dB re 1 µPa) |
|---|---|---|---|---|---|---|---|---|
| 15:39:01 | 1 | 10 | 224.55 | 205.38 | 231.01 | 231.01 | 224.46 | 234.36 |
| 15:44:14 | 4 | 39 | 204.47 | 190.76 | 213.53 | 213.53 | 210.19 | 218.04 |
| 15:49:10 | 5 | 46 | 199.43 | 186.51 | 206.38 | 206.38 | 205.63 | 212.03 |
| 15:55:32 | 3 | 39 | 205.42 | 191.81 | 214.90 | 214.90 | 211.93 | 219.56 |
| 16:00:39 | 2 | 24 | 215.25 | 199.89 | 222.53 | 222.53 | 222.11 | 228.34 |
| 16:11:09 | 4 | 39 | 204.94 | 191.29 | 214.16 | 214.16 | 211.21 | 218.83 |
| 16:51:21 | 3 | 39 | 205.58 | 191.95 | 215.10 | 215.10 | 212.00 | 219.71 |
| 16:55:55 | 5 | 47 | 199.55 | 186.69 | 206.14 | 205.99 | 206.14 | 212.09 |
| 17:01:16 | 2 | 24 | 215.13 | 199.88 | 222.64 | 222.64 | 222.26 | 228.47 |
| 17:09:14 | 1 | 10 | 225.49 | 205.75 | 232.01 | 232.01 | 225.38 | 235.33 |
| 17:15:57 | 1 | 10 | 224.82 | 205.63 | 231.45 | 231.45 | 224.86 | 234.78 |
| 17:22:00 | 3 | 38 | 206.54 | 192.76 | 215.99 | 215.99 | 213.77 | 220.97 |
| 17:28:03 | 5 | 45 | 199.98 | 186.01 | 205.45 | 205.45 | 205.00 | 211.25 |
| 17:38:22 | 2 | 21 | 215.86 | 200.03 | 222.61 | 222.48 | 222.61 | 228.57 |
| 17:43:12 | 4 | 38 | 205.23 | 191.55 | 214.41 | 214.41 | 211.25 | 218.99 |
| 17:53:57 | 5 | 46 | 199.24 | 186.24 | 205.76 | 205.76 | 205.70 | 211.75 |
| 17:58:32 | 2 | 24 | 215.18 | 199.96 | 222.35 | 222.35 | 222.17 | 228.28 |
| 18:04:53 | 4 | 39 | 204.87 | 191.23 | 214.06 | 214.06 | 210.59 | 218.52 |
| 18:10:20 | 3 | 39 | 206.16 | 192.49 | 215.62 | 215.62 | 212.98 | 220.42 |
| 18:21:55 | 1 | 10 | 225.12 | 205.87 | 231.69 | 231.69 | 225.09 | 235.02 |
| 18:26:38 | 5 | 46 | 199.12 | 186.22 | 205.62 | 205.62 | 205.51 | 211.58 |
| 18:38:40 | 4 | 39 | 204.95 | 191.31 | 214.16 | 214.16 | 210.27 | 218.45 |
| 18:45:17 | 3 | 39 | 205.98 | 192.32 | 215.57 | 215.57 | 212.18 | 220.06 |
| 18:51:03 | 2 | 21 | 216.12 | 200.22 | 223.13 | 223.13 | 222.75 | 228.96 |

Table 4. Shot summary metrics for walleye on 15 Sep 2012.

| Time (UTC) | Cage | Duration (ms) | rms SPL (dB re 1 µPa) | SEL (dB re 1 µPa$^2$·s) | Peak SPL (dB re 1 µPa) | Peak+ SPL (dB re 1 µPa) | Peak− SPL (dB re 1 µPa) | Peak-Peak SPL (dB re 1 µPa) |
|---|---|---|---|---|---|---|---|---|
| 16:47:01 | 5 | 46 | 198.33 | 186.00 | 204.63 | 204.63 | 204.53 | 210.60 |
| 16:53:47 | 2 | 25 | 215.12 | 200.01 | 223.20 | 221.88 | 223.20 | 228.59 |
| 17:17:11 | 1 | 10 | 224.88 | 205.58 | 230.99 | 230.99 | 224.40 | 234.33 |
| 17:23:25 | 4 | 38 | 205.37 | 192.12 | 214.68 | 214.68 | 211.09 | 219.09 |
| 17:30:04 | 3 | 38 | 205.86 | 192.61 | 215.45 | 215.45 | 211.82 | 219.84 |
| 17:36:06 | 1 | 9 | 224.85 | 205.51 | 230.99 | 230.99 | 224.48 | 234.35 |
| 17:40:37 | 5 | 43 | 199.91 | 187.27 | 207.81 | 207.81 | 206.39 | 213.15 |
| 17:48:26 | 4 | 38 | 205.56 | 192.26 | 214.73 | 214.73 | 210.99 | 219.08 |
| 17:54:50 | 3 | 38 | 205.97 | 192.69 | 215.49 | 215.49 | 212.04 | 219.95 |
| 18:14:11 | 2 | 25 | 215.80 | 200.77 | 223.25 | 222.33 | 223.25 | 228.82 |
| 20:09:06 | 4 | 38 | 205.18 | 191.92 | 214.70 | 214.70 | 210.52 | 218.88 |
| 20:14:27 | 5 | 45 | 199.47 | 186.91 | 205.93 | 205.93 | 205.59 | 211.78 |
| 20:20:53 | 1 | 10 | 224.95 | 205.74 | 231.09 | 231.09 | 224.34 | 234.37 |
| 20:28:20 | 2 | 24 | 217.47 | 202.16 | 224.97 | 224.43 | 224.97 | 230.73 |
| 20:33:11 | 3 | 34 | 207.72 | 193.97 | 216.11 | 216.11 | 213.20 | 220.79 |
| 20:40:37 | 1 | 16 | 222.19 | 205.13 | 229.91 | 229.91 | 224.42 | 233.61 |
| 20:14:25 | 3 | 38 | 205.79 | 192.52 | 215.44 | 215.44 | 211.71 | 219.79 |
| 20:55:45 | 4 | 38 | 204.94 | 191.70 | 214.12 | 214.12 | 210.66 | 218.58 |
| 21:01:27 | 2 | 20 | 217.68 | 201.60 | 224.63 | 224.58 | 224.63 | 230.62 |
| 20:33:11 | 3 | 34 | 207.72 | 193.97 | 216.11 | 216.11 | 213.20 | 220.79 |
| 21:20:03 | 2 | 21 | 216.86 | 201.00 | 224.05 | 223.01 | 224.05 | 229.56 |
| 21:25:06 | 4 | 38 | 205.58 | 192.27 | 214.85 | 214.85 | 210.87 | 219.11 |
| 21:32:02 | 5 | 43 | 199.75 | 187.13 | 207.85 | 207.85 | 205.70 | 212.86 |
| 21:38:19 | 1 | 12 | 223.60 | 205.34 | 230.26 | 230.26 | 223.94 | 233.69 |
| 21:50:51 | 2 | 26 | 214.51 | 199.59 | 223.34 | 223.34 | 218.49 | 227.27 |

Table 5. Control exposure summary metrics for paddlefish starting 13 Sep 2012 (UTC).

| Time (UTC) | Duration (mm:ss) | rms SPL (dB re 1 µPa) | SEL (dB re 1 µPa$^2$·s) | Peak SPL (dB re 1 µPa) | Peak+ SPL (dB re 1 µPa) | Peak− SPL (dB re 1 µPa) | Peak-Peak SPL (dB re 1 µPa) |
|---|---|---|---|---|---|---|---|
| 22:13:49 | 02:50 | 98.95 | 120.49 | 144.51 | 144.51 | 143.18 | 149.89 |
| 23:16:12 | 01:32 | 101.49 | 121.50 | 145.51 | 143.17 | 145.51 | 150.44 |
| 00:02:28 | 01:32 | 110.38 | 126.82 | 145.38 | 145.38 | 143.85 | 150.67 |

Table 6. Control exposure summary metrics for pallid sturgeon, 14 Sep 2012 (UTC).

| Time (UTC) | Duration (mm:ss) | rms SPL (dB re 1 µPa) | SEL (dB re 1 µPa$^2$·s) | Peak SPL (dB re 1 µPa) | Peak+ SPL (dB re 1 µPa) | Peak− SPL (dB re 1 µPa) | Peak-Peak SPL (dB re 1 µPa) |
|---|---|---|---|---|---|---|---|
| 16:06:03 | 00:54 | 104.89 | 122.20 | 147.56 | 147.56 | 147.49 | 153.55 |
| 16:57:39 | 02:04 | 96.81 | 117.73 | 135.05 | 131.69 | 135.05 | 139.55 |
| 17:34:26 | 01:06 | 111.22 | 129.38 | 138.45 | 138.30 | 138.45 | 144.40 |
| 18:11:18 | 01:14 | 101.79 | 120.51 | 134.27 | 134.27 | 132.01 | 139.23 |
| 18:27:16 | 02:25 | 108.50 | 130.13 | 152.73 | 152.73 | 151.83 | 158.31 |

Table 7. Control exposure summary metrics for walleye, 15 Sep 2012 (UTC).

| Time (UTC) | Duration (mm:ss) | rms SPL (dB re 1 µPa) | SEL (dB re 1 µPa$^2$·s) | Peak SPL (dB re 1 µPa) | Peak+ SPL (dB re 1 µPa) | Peak− SPL (dB re 1 µPa) | Peak-Peak SPL (dB re 1 µPa) |
|---|---|---|---|---|---|---|---|
| 16:56:18 | 02:11 | 108.57 | 129.75 | 143.53 | 143.53 | 141.26 | 148.49 |
| 18:01:20 | 01:25 | 106.59 | 125.90 | 134.30 | 132.60 | 134.30 | 139.51 |
| 20:04:35 | 00:33 | 105.92 | 121.15 | 125.36 | 125.36 | 124.55 | 130.99 |
| 21:09:33 | 00:43 | 110.35 | 126.65 | 128.32 | 128.32 | 128.23 | 134.29 |
| 21:40:35 | 01:34 | 107.92 | 127.68 | 136.10 | 136.10 | 133.25 | 140.81 |

## Acknowledgements

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## Literature Cited

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# APPENDIX D

## Necropsy Protocol

# Fish Handling and Necropsy Protocols (Including Training)

## Training and Other Issues to Discuss Prior to Start of Study

1. Necropsy Training
    a. Discuss safety issues. As needed, modify procedures after the discussion.
    b. Provide a general introduction and discussion about what we will be doing, i.e., philosophy for necropsy.
    c. Discuss general necropsy methodologies and approaches.
    d. Review the types of effects (damage) likely to be encountered in the necropsies (Table).
        i. Show images of typical damage for sturgeon and examples from other species
        ii. Discuss other types of effects that might be encountered.
        iii. Following discussion, modify tables as needed.
    e. Review procedures for use of MS-222, human safety, and disposal of materials. If needed, modify procedures after discussion.
    f. Practice preparation of MS-222, euthanasia, and dissection of several fish
    g. Discuss necropsy procedures based on practice dissections, and modify procedures as appropriate to make them more effective.
    h. Discuss photography of necropsied fish, including when and how to do the photographs, and labeling of photographs.
2. Data sheets and data recording
    a. Develop actual data sheets and Excel data files.
    b. Discuss how to actually enter data and other data management issues.
    c. Discussion of review/Q.C. of data and one over of data (assurance of data accuracy).
    d. Discussion of importance of doing “blind” experiments so individuals doing necropsy do not know exposure regimes of animals being examined.
    e. Labeling data in the computer ands torage of printed data sheets.
    f. Procedures for backup of data.
3. Pit tag data and data sheets
    a. Information to be recorded in data sheets after tagging (e.g., tag number, fish size, fish condition)
    b. How to properly read pit tags and use of the pit tag reader.
    c. Entering pit tag information into the computer database.
4. Answer questions and modify all procedures as needed based on discussion.

## MS-222, Waste Handling, Disposal of Tissues, Safety

1. Preparation of MS-222 solution[10]
   a. MS-222 is only to be handled by persons wearing disposable gloves, safety goggles, and a safety mask. The mask may be removed after the solution is mixed.
   b. Unless a hood is available, take the required amount of MS-222 from the container out of doors or, if windy, in a protected outside area.
   c. Put MS-222 into the appropriate sized water container.
   d. Use a stick to mix the MS-222
   e. MS-222 doses for mortality were as follows:
      i. Paddlefish – 300 mg/L
      ii. Sturgeon – 250 mg/L
      iii. Walleye – 200 mg/L – 250 mg/L

      MS-222 Container should be labeled so others will not use it.
   f. An MS-222 solution can be used for multiple fish during a day. Once the water quality drops or MS-222 begins to lose its effectiveness, dispose of the MS-222 solution.
      i. MS-222 has designated buckets out of high traffic areas, and at the close of the project a hazard team picks up MS-222 for disposal.
2. Disposal of tissue
   a. Collect all tissue from each fish dissected, even small pieces.
   b. Place into plastic bag and seal bag.
   c. Place bag in designated refrigerator, then at the close of each day fish carcasses and remains are taken to a disposal site designated by Game and Fish for burial.
3. Safety procedures
   a. All fish handling and handling of MS-222 will be done wearing disposable gloves and goggles. There are no exceptions. Gloves are available that are latex or hyper-allergenic.
      i. If gloves rip, replace them immediately.
      ii. Following use, dispose of gloves in designated trash pin
   b. All sharps (blades, glass, etc.) must be disposed of in the approved “sharps” container.
      i. At the end of the study, the sharps container is to be disposed of at the appropriate site or given to the hatchery for their use if not full.
      ii. Nothing sharp should be placed anywhere but in the sharps container. There is no exception!!!

[10] Tricaine Methanesulfonate (MS-222) (Western Chemical Inc. Ferndale WA USA, http://www.wchemical.com/TRICAINE-S-MS-222-P43C7.aspx).

## Tagging and Acquisition of Data During Exposure Studies

1. Tags (PIT tags for pallid sturgeon and adult walleye and Floy tags for paddlefish) will be inserted approximately 7 days before start of experiments in the dorsal musculature. For YOY walleye tagging to be done the day of the experiment and involve fin clips since the fish are too small to carry tags.
    a. Use data sheets to gather all of the data about fish as they are tagged (tag number, size, mass, etc.).
    b. Enter data regarding tag in waterproof notebook as each fish is tagged and in a final data sheet when the necropsy is completed.
    c. The tag number is the only identifying number to be used for each fish.
2. Pit tag information spreadsheet. (Note, this is distinct and separate from the necropsy data file.)
    a. The individuals doing necropsies should not be able to see this file at any time.
    b. File should include pit tag number, date inserted, species, fish length, treatment group.
    c. The first part of the data should be entered when pit tag is inserted into the fish.
    d. As fish are used in a treatment group the additional data needs to be added by designated fish handlers.
    e. Data on pit tag entry must be completed when the fish have pit tag inserted, as well as when they are assigned to treatment group.
    f. Tagging and tag-reading done on fish without anesthesia. Care should be taken of the fish if they are moving or thrashing around.
    g. A hard copy of the pit tag sheet is provided to the person on the boat for entry of information about each fish including the information about the experiment and cage number.
    h. At end of the whole study, the necropsy sheets and the pit tag sheet are provided to one individual who will add the fish treatment group to the necropsy sheet and then sort by treatment.
3. Once fish are moved to the boat for sound exposure and as they are put into their experimental exposure cage, additional data (dissolved oxygen [DO] levels and temperatures) are to be recorded in the waterproof notebook and later transferred to electronic data sheets:
    a. Pit tag is read by the fish handler and confirmed by a second person.
    b. The experiment to which the fish is assigned is entered into the sheet.
    c. The cage in which the fish is placed is indicated (1 closest to the source, 6 control).
    d. Within the 48 h of an exposure experiment, the person conducting the data recording will enter the information into the PC from a given experiment and for all fish.
    e. A 2$^{nd}$ person Q.C's the data entered to make sure it is correctly entered into the electronic data sheet.
    f. If the electronic data does not match the information on the data sheet the data manager has to confirm the difference and approves any change.
    g. All data sheets are to be put in a folder and held in the necropsy room, with photocopies held at the hotel.

# Necropsy Procedures

1. Dealing with dead fish:
    a. All fish will be euthanized and chilled before necropsy (see MS-222 preparation below).
    b. Once fish are euthanized (see procedures), they will be gently dried with paper towels (taking care not to damage the surface or remove scales), wrapped in dry paper, and put into a refrigerator at a temperature of about 38 $^{o}$F. Prior to fish being put into the chiller the time and day of death should be noted and put the paper in which the fish is wrapped for chilling.
    c. Care must be taken not to drop, rub, or bend the fish.
    d. For fish that died prior to euthanization, note the time fish was found (or died) on the paper in which the fish was wrapped, along with an estimate of time of death or time between when fish was found, and its condition.
    e. When it is time to necropsy a particular fish, remove fish from refrigerator, note the information about the euthanization written on the paper and transfer to the data sheets for the necropsy, and then carefully remove from paper and perform the necropsy.
2. Data sheets and data entry into Excel data sheets
    a. Fill out a separate paper data sheet for each fish (note, use pencil or indelible pen only!).
    b. Enter all required information in the data sheets, including the tag number. Since the tag number is so critical, read several times. A second person should read the same tag and confirm that the number is correct.
    c. Fill in all data about fish, including euthanization information from the paper in which the fish were wrapped for chilling.
3. During necropsy:
    a. If there is any damage to a structure that is being examined enter a “1” into the data sheet at that characteristic.
    b. After fish has been fully examined go back over the data sheet to make sure that all information is filled in.
    c. MAKE SURE ALL WRITING IS LEGIBLE AND CLEAR!
    d. After entering data into computer (see below) put the data sheet into data folder (kept at fish hatchery in necropsy room, with copies held at hotel.
4. Notes:
    a. Any additional observations (e.g., something not normally seen, other special issues) are to be noted in "comments" section of the data sheet. In addition, use the comment section to discuss any observations on extent of damage seen.
    b. Keeping careful and complete notes is essential. It is expected that notes will be made in the “comments” section about most animals necropsied.
5. Data entry into Excel data sheet:
    a. Within a week after necropsy scan, copy, and enter the data from the sheet into the computer.

b. All information is held in one data file.
c. Data is checked by a separate person.
d. Be sure and save the file after data is entered into the computer.
e. Check the entered data against the hand-written data sheet item by item to make sure entry is correct. Correct if necessary
f. At very end, save file one more time.

6. Data storage and backup:
    a. At the end of the day, all data acquired to that point must be backed up to a separate hard disk and server, including to the Continental Shelf Associates (CSA) server (off site).
    b. CSA will arrange that each day's data is backed up as a discrete file and saved separately.
7. Euthanasia (gloves, plastic apron, safety glasses to be used)
    a. Get MS-222 container from designated storage location (that is out of the way of people walking by).
    b. Place fish into labeled bucket of MS-222.
    c. Leave fish in MS-222 until it turns upside down and stops all opercular movement for at least 10 min.
    d. Remove fish from MS-222.
    e. Gently dry body of fish with paper towels.
    f. Wrap in paper, label, and place in a refrigerator
    g. Before necropsy, put MS-222 container into designated place and put cover on the container.
8. Numbering of fish
    a. In the data sheets, each fish is to be numbered the same as its tag.
    b. This number will be the file number for figures, and the identifier in the necropsy data table.
    c. Tags will be read prior to necropsy.
    d. The necropsy data sheet will contain additional information about the fish.
9. Numbering of fish and exposures
    a. Information about the exposure parameters for each fish is maintained in the tag number Excel file
    b. As a fish is placed in a cage its pit tag number is read and recorded.
    c. The following information is also to be entered into the table by that tag:
        i. Experiment number – each exposure is a separate letter. The first, on the first morning, is "A." The next exposure is "B," etc.
        ii. Cage number starts with 1, closest to the source, 2 is the next distance from the source, 5 the furthest distance exposed, and 6 is the control. Thus for pallid sturgeon
10. Photography and numbering
    a. Photographs should be taken during necropsy. These should be taken as close to the structure being photographed as possible using the zoom lens on the camera, but making sure the image is in focus.

b. First photo for each fish should include:
    i. A ruler for size indications; and
    ii. A label (hand written) with the fish tag number.
c. Subsequent photos should be made as close to the object as possible, but if a large area try and keep the fish number label in the picture without interfering with the object being photographed.
d. Even if no damage is seen, take one or two pictures of the internal structures for "normal" comparison.
e. Photograph every structure that shows any effect. If necessary, take several photos of each structure.
f. After finishing a necropsy, and after entering data into computer, download the pictures from that fish to a photo directory that has the tag number of the fish as its name.
    i. NOTE the camera-designated numbers on the pictures and put the range of those numbers into the data spreadsheet for the fish.
    ii. Put the file name for the photos in the spreadsheet for the fish

11. One over and review of data
    a. At the end of each data designated personnel assembles all of the data sheets from that day.
    b. Within a week designated personnel enters the data from necropsy sheets.
    c. The Individuals doing necropsies go over the entered data for all fish assigned to ensure that the entries are correct and complete.
    d. If there are discrepancies between the data sheet and the entered data, the data manager has to review, correct, and approve the changes. The correct number, from the data sheet, is then entered into the computer.

## Additional Notes

- A fish is considered dead when the opercular movement has stopped and the fish does not respond to stimuli. Opercular movement has to stop for minimum of 10 min.
- Monitor fish being held so the most accurate time of morbidity or death is recorded.
- Fish tanks are checked once in the morning and once in the evening for mortalities.
- All feeding stopped one day prior to tagging. Fish were not fed any time after tagging.
- All data entry onto data sheets and in notebooks should use a pencil or indelible pen to prevent data loss of the paper gets wet.

## Internal Signs of Injury

A ventral cut from the anal pore up to the pericardial region will be made to expose all internal organs of interest. Cut parallel to the body through the ventral musculature but not too deep in order to prevent damage to the internal organs by the scissors. Insert the ball-point scissors into the vent to begin the incision. When cutting, apply a slight outward pressure with the ball point scissors 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 by 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.

Upon exposing the internal organs, it is important to initially look for any signs of blood that would suggest an internal injury.

**Swim bladder** – Move all the internal organs (stomach, intestine, liver, any fat, etc.) to the side to expose the entire 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 presence and location of a perforation.

**Kidney** – Removal or cutting open the swim bladder will reveal 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.

**Table: List of All Potential Internal Injuries**

| |
|---|
| Body muscles hematoma |
| Burst capillaries along kidneys and wall |
| Fully deflated swim bladder (no ruptures) |
| Heart beating upon opening |
| Partially deflated swim bladder (no ruptures |
| Renal (kidney) anterior embolism |
| Renal (kidney) hemorrhage |
| Renal hematoma |
| Renal mid embolism |
| Renal posterior embolism |
| Ruptured swim bladder anterior |
| Ruptured swim bladder mid |
| Ruptured swim bladder posterior |
| Swim bladder hematoma |

# APPENDIX E

## Data Sheets

**Fish Sound Exposure Sheet**. Used to record the signal levels to which each fish was exposed. This is an example for a sheet for pallid sturgeon (PS). The actual sheet would provide space for all fish used. A similar sheet was used for the other species.

| Date | Species | Treatment | Block | Rep. | Dist. (m) | Depth (m) | SPL (dB) | SEL (dB) | Time of Shot | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| | PF | | 5 | 1 | | | | | | |
| | PS | | 1 | 1 | | | | | | |
| | PS | | 6 | 1 | | | | | | |
| | PS | | 2 | 1 | | | | | | |
| | PS | | 4 | 1 | | | | | | |
| | PS | | 3 | 1 | | | | | | |
| | PS | | 7 | 1 | | | | | | |
| | PS | | 4 | 2 | | | | | | |
| | PS | | 6 | 2 | | | | | | |
| | PS | | 2 | 2 | | | | | | |
| | PS | | 5 | 2 | | | | | | |
| | PS | | 7 | 2 | | | | | | |
| | PS | | 3 | 2 | | | | | | |
| | PS | | 1 | 2 | | | | | | |
| | PS | | 7 | 3 | | | | | | |

**Pallid Sturgeon Fish Handling Data Sheet**. The full data sheet has room for all fish used. A similar sheet was used for each species.

**Pallid Sturgeon Study**

| | Date | Species | Tag # | Treatment | Rep. | Block | Dist. (m) | Depth (m) | Start Time | End Time | DO | Temp. | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | PS | | 1 | 1 | 1 | | | | | | | |
| 2 | | PS | | 1 | 1 | 1 | | | | | | | |
| 3 | | PS | | 1 | 1 | 1 | | | | | | | |
| 4 | | PS | | 1 | 1 | 1 | | | | | | | |
| 5 | | PS | | 4 | 1 | 2 | | | | | | | |
| 6 | | PS | | 4 | 1 | 2 | | | | | | | |
| 7 | | PS | | 4 | 1 | 2 | | | | | | | |
| 8 | | PS | | 4 | 1 | 2 | | | | | | | |
| 9 | | PS | | 5 | 1 | 3 | | | | | | | |
| 10 | | PS | | 5 | 1 | 3 | | | | | | | |
| 11 | | PS | | 5 | 1 | 3 | | | | | | | |
| 12 | | PS | | 5 | 1 | 3 | | | | | | | |
| 13 | | PS | | 3 | 1 | 4 | | | | | | | |

**Pallid Sturgeon General Data Sheet**. Example for several fish. A similar data sheet was used for all species.

4 fish per cage. Treatment type= what distance fish will be placed at.

| Date | Species | Treatment Type | Rep. | Block |
|---|---|---|---|---|
| | PS | 1 | 1 | 1 |
| | PS | 4 | 1 | 2 |
| | PS | 5 | 1 | 3 |
| | PS | 3 | 1 | 4 |
| | PS | 2 | 1 | 5 |
| | PS | 6 | 1 | 6 |
| | PS | 4 | 2 | 7 |
| | PS | 3 | 2 | 8 |
| | PS | 5 | 2 | 9 |
| | PS | 6 | 2 | 10 |
| | PS | 2 | 2 | 11 |
| | PS | 1 | 2 | 12 |

**Necropsy sheet.**

| Date | Scientist Name: | Species | Tag # | Tag Recovered (Y / N) |
|---|---|---|---|---|
| Length (TL/ FL/ SL) (mm) | Weight (g): | Camera #: | Total # Photos: | |
| Data Entered By: | Data QA/QC'd By: | Refrigeration Time: | | |

## Internal Observations

| | | | | | Swim Bladder | | | | | | Kidney | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Heart beating upon opening | Fat Hematoma | Body muscles Hematoma | Burst capillaries along kidneys and wall | Ruptured SB anterior | Ruptured SB mid | Ruptured SB posterior | Bruised Swim bladder | Partially deflated (no ruptures) | Fully deflated (no ruptures) | Kidney rupture | Renal (kidney) hemorrhage | Renal (kidney) Hematoma | Renal (kidney) anterior embolism | Renal (kidney) mid embolism | Renal (kidney) posterior embolism |
| **Y or --** | | | | | | | | | | | | | | | | |

**Comments:**

| Data Entered By: | Data QA/QC'd By: |
|---|---|

# APPENDIX F

## Waste Disposal

## MS-222

Euthanization for necropsy was done using an overdose of Tricaine Methanesulfonate (MS-222). Once the water quality of a batch of MS-222 decreased or its effectiveness in euthanization decreased (see **Appendix D**) the solution was disposed of into fifty-five gallon drums that were labeled MS-222. The drums had a snap ring along the top rim that allowed for tightening the lid. The drums were located near the maintenance building at the Garrison Fish Hatchery. This location was chosen so that in the case of accidental spillage the MS-222 would not go down the drains. As a result of euthanizing all of the fish, a total of five, fifty-five gallon drums of MS-222 were generated. The drums were picked up on 27 September 2012 by Clean Harbors, a licensed hazard waste disposal company. Troy Brunsell, Hess Health, Safety and Environment Manager made arrangements for the disposal.

## Fish

A total of 939 fish were disposed of after completion of this study:

370 Paddlefish
318 Sturgeon
150 Adult Walleye
101 Young of year Walleye

After necropsy fish carcasses' were put into plastic bags for transport to the disposal site which was located on the Garrison Dam National Fish Hatchery Complex. A pit five foot by three foot deep was dug using a backhoe in an area closed to the public. The fish were moved from the plastic bags into the pit throughout the study and at the conclusion of the study the pit covered with two feet of dirt. Rob Holm (USFWS), Garrison Dam National Fish Hatchery Complex manger supervised this effort.

# APPENDIX G

## Statistical Analysis

# The Analysis of the Effects of Seismic Surveys on Pallid Sturgeon, Paddle Fish, and Walleye

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25 October 2012

## Analysis Methods

The experimental units in the study were the individual cages with multiple fish inside. Each cage represents a binomial sample of $n_i$ fish, of which $x_i$ died or had mortal injury. There were five distance classes from the sound source, with each cage having separate measures of sound exposure. Two sound covariates were used as independent variables to assess the relationship between sound level and death/mortal injury. These were negative peak pressure (i.e., PEAK_) and sound exposure level (SEL). There were also control cages that were placed in the water at a “safe” distance. There were observed death/mortal injury among the control fish, so an Abbott’s adjustment (Finney 1971:125) to the treatment fish was necessary.

The Abbott’s adjustment (Finney 1971:125) is based on the assumption that surviving handling (i.e., control survival) is independent of surviving the treatment, such that

$$E(S_i) = S_C S_{T_i},$$

where

$S_i$ = observed survival of test fish exposed to handling and treatment $i$,

$S_C$ = probability of surviving handling (i.e., control survival),

$S_{T_i}$ = probability of survival for fish exposed to a treatment $i$.

Data analysis was therefore based on numbers of test fish that were alive and healthy (i.e., $n_i - x_i$) using generalized linear models (McCullagh and Nelder 1989) with a binomial error structure and log-link. Analysis of deviance (ANODEV) was used to test hypotheses based on the cage data.

The analysis tested several hypotheses concerning the relationship between sound exposure from the air guns and fish mortality/mortal injury $(M)$. These hypotheses included:

6. $H_o$: $M_c = M_T$ vs. $H_a$: $M_C \neq M_T$ — Control mortality/injury same as pooled treatments

7. $H_o$: $M_c = M_i \quad \forall i$ vs. $H_a$: $M_C \neq M_i \quad \forall i$ — Control mortality/injury same as each treatment

8. $H_o$: $M_c \geq M_{T_i}$ vs. $H_a$: $M_C < M_{T_i}$ — Control mortality/injury $\geq$ $i$th treatment

9. $H_o$: $M \neq f(\text{PEAK\_})$ vs. $H_a$: $M = f(\text{PEAK\_})$ — Mortality/injury not a function of PEAK_

10. $H_o$: $M_i \neq f(\text{SEL})$ vs. $H_a$: $M_i = f(\text{SEL})$ — Mortality/injury not a function of SEL

Contingency tables and data plots were also used to summarize the test results. Estimates of death/mortal injury pooled across cages $(M_{T_i})$ within a distance class were plotted against average exposure levels. The empirical estimates of death/mortal injury (i.e., $\tilde{M}_{T_i}$) were corrected for the death/mortal injury rate observed across the pooled controls (i.e., $M_C$) according to the Abbott formula where

$$S_i = S_C S_{T_i}$$

or

$$M_{T_i} = \frac{\hat{M}_{T_i} - M_C}{1 - M_C}, \quad (2)$$

where $M_T$ is the control adjusted mortality/injury probability. For the summaries, data were pooled across replicate cages because sample sizes in the individual cages were too small to convey trends in the mortality data.

# Results

## Pallid Sturgeon

In the acoustic trials of pallid sturgeon, no 7-day mortalities occurred, but there were fish with mortal injuries. There were also control fish with mortal injuries (Attachment A). Consequently, the analysis of the acoustic trails required adjustment for control effects.

An R x C contingency table (Table 1) displays the raw counts for the five different distance classes plus controls, after pooling across replicates. The observed proportion of fish with mortal injury among the treatments ranged from 0.0833–0.2143. The control fish had an observed proportion of 0.1538 with mortal injury. Pooling across the five treatment levels, the observed proportion of mortal injuries was 0.1549, nearly identical to the control rate. The R x C contingency table found no difference in proportions with mortal injury among the six groups of fish $\left(P\left(\chi_5^2 \geq 1.1893\right) = 0.9461\right)$.

Analysis of deviance (ANODEV) found no difference in the rate of mortal injury between the control and treatments pooled $\left(P\left(F_{1,27} \geq 0.0001\right) = 0.9924\right)$ or individually $\left(P(F \geq 0.2047) = 0.9572\right)$ (Table 2).

In addition, none of the five test groups had significantly higher rates of mortal injury than the controls $(P \geq 0.3554)$ (Table 2).

The ANODEV was also used to test whether there was a significant relationship between the level of sound exposure and the rate of mortal injury. There was no relationship found between the level of negative peak pressure (PEAK_) and the rate of mortal injury (*P* = 0.9987), nor between sound exposure level (SEL) and the rate of mortal injury (*P* = 0.9914) (Table 2). Plots of the observed rates of mortal injury after correcting for controls illustrate no pattern with regard to PEAK_ or SEL (Figure 1).

The results of these analyses suggest at the sound exposure levels tested, there was no effect on mortality or mortal injury to pallid sturgeon.

## Paddle Fish

An R x C contingency table (Table 3) displays the raw counts for the five different distance classes plus controls after pooling across replicates (Attachment B). The observed proportions of fish with mortal injuries (no mortalities observed) among the treatments ranged from 0.0 – 0.3636 in a nonmonotonic pattern. The overall proportion of mortal injuries among treatment fish was 0.16. The control fish had an observed proportion of 0.10 with mortal injury. The R x C contingency table found no differences in proportions with mortal injury among the six groups of fish $\left(P\left(\chi_5^2 \geq 6.5062\right) = 0.2600\right)$.

ANODEV found no difference in the rate of mortal injury between the controls and all treatments pooled $\left(P\left(F_{1,16} \geq 0.1775\right) = 0.6791\right)$ or individually $\left(P\left(F_{5,16} \geq 1.0829\right) = 0.4176\right)$ (Table 4). In addition, none of the five test groups had significantly high rates of mortal injury than the controls ($P \geq 0.1167$) (Table 2). The only test group that approached significantly more mortal injury was the most proximate group, treatment 1 (*P* = 0.1167) with an observed proportion of 0.3636.

The ANODEV was also used to test whether there was a relationship between the level of sound exposure and the rate of mortal injury. Neither negative peak energy (PEAK_) (*P* = 0.6230) nor sound exposure level (SEL) (*P* = 0.6077) was related to the rate of mortal injury. Tests of positive relationships would have *P*-values of 0.3115 and 0.3039, respectively. Plots of the observed rates of mortal injury corrected for controls illustrate no definitive pattern with regard to PEAK_ or SEL (Figure 2).

The results of these analyses provide no definitive evidence of increased mortality or mortal injury to paddle fish at the exposure level tests. There is marginal evidence (*P* = 0.1167) that there might be possible elevated rates of mortal injury at the closest treatment level 1 with average PEAK_ of 223.3. The small sample sizes make determining significant effects difficult at the individual treatment level.

Table 1. Counts of observed mortal injury by treatment group (proportion in parentheses) for pallid sturgeon. Distance classes 1 through 5 are in order of increasing distance from sound source. Chi-square test of homogeneity was nonsignificant $\left(P\left(\chi_5^2 \geq 1.1873\right) = 0.9461\right)$.

| | Distance class | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Control |
| Alive & healthy | 11 (0.9167) | 11 (0.7857) | 13 (0.8667) | 12 (0.8000) | 13 (0.8667) | 11 (0.8462) |
| Mortal injury | 1 (0.0833) | 3 (0.2143) | 2 (0.1333) | 3 (0.2000) | 2 (0.1333) | 2 (0.1538) |

Table 2. Summary of results from the ANODEV of mortal injury data from the pallid sturgeon experiment with null hypotheses tested, test statistics, and associated *P*-values.

| Null hypotheses | Test statistic | *P*-value |
|---|---|---|
| $\mathrm{H_o}$: $M_C = \bar{M}_T$ | $F_{1,27} = 0.0001$ | 0.9924 |
| $\mathrm{H_o}$: $M_C = M_{T_i}; i = 1, \ldots, 5$ | $F_{5,27} = 0.2047$ | 0.9572 |
| $\mathrm{H_o}$: $M_C \geq M_{T_1}$ | $Z = -0.4991$ | 0.6912 |
| $\mathrm{H_o}$: $M_C \geq M_{T_2}$ | $Z = 0.3709$ | 0.3554 |
| $\mathrm{H_o}$: $M_C \geq M_{T_3}$ | $Z = -0.1408$ | 0.5560 |
| $\mathrm{H_o}$: $M_C \geq M_{T_4}$ | $Z = 0.2934$ | 0.3846 |
| $\mathrm{H_o}$: $M_C \geq M_{T_5}$ | $Z = -0.1408$ | 0.5560 |
| $\mathrm{H_o}$: $M_{T_i} \neq f(\mathrm{PEAK\_})$ | $F_{1,27} = 0.0000$ | 0.9987 |
| $\mathrm{H_o}$: $M_{T_i} \neq f(\mathrm{SEL})$ | $F_{1,27} = 0.0001$ | 0.9914 |

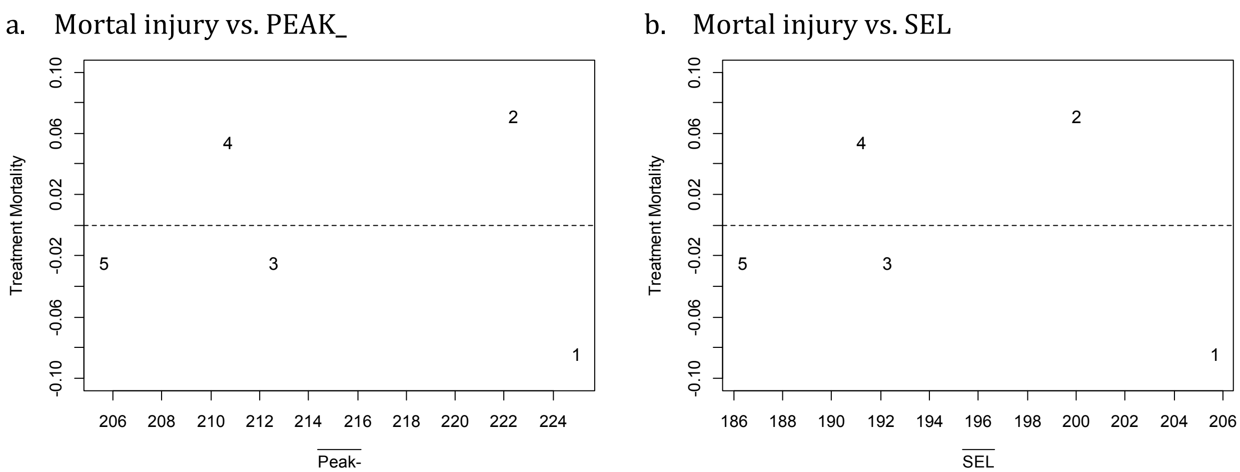



Figure 1. Scatterplots of observed rates of mortal injury after corrections for control rates against (a) peak negative sound pressure and (b) sound exposure level. Data were pooled over replicates and exposure levels averaged. Treatments 1–5 are in order of increasing distance from sound source.

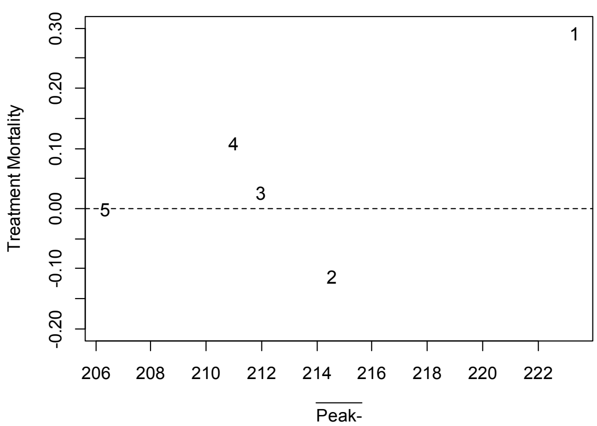
Table 3. Counts of observed mortal injury by treatment group (proportion in parentheses) for paddle fish. Distance classes 1 through 5 are in order of increasing distance from sound source. Chi-square test of homogeneity was nonsignificant $\left(P\left(\chi_5^2 \geq 6.5062\right) = 0.2600\right)$.

| | Distance class | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Control |
| Alive & healthy | 7 (0.6364) | 11 (1.0000) | 7 (0.8750) | 8 (0.8000) | 9 (0.9000) | 9 (0.9000) |
| Mortal injury | 4 (0.3636) | 0 (0.0000) | 1 (0.1250) | 2 (0.2000) | 1 (0.1000) | 1 (0.1000) |

Table 4. Summary of results from the ANODEV of mortal injury data from the paddle fish experiment with null hypotheses tested, test statistics, and associated *P*-values.

| Null hypotheses | Test statistic | *P*-value |
|---|---|---|
| $H_o$: $M_C = \bar{M}_T$ | $F_{1,16} = 0.1775$ | 0.6791 |
| $H_o$: $M_C = M_{T_i}$; $i = 1, \ldots, 5$ | $F_{5,12} = 1.0829$ | 0.4176 |
| $H_o$: $M_C \geq M_{T_1}$ | $Z = 1.1916$ | 0.1167 |
| $H_o$: $M_C \geq M_{T_2}$ | $Z = -0.8629$ | 0.8059 |
| $H_o$: $M_C \geq M_{T_3}$ | $Z = 0.1429$ | 0.4432 |
| $H_o$: $M_C \geq M_{T_4}$ | $Z = 0.5351$ | 0.2963 |
| $H_o$: $M_C \geq M_{T_5}$ | $Z = 0.0000$ | 0.5000 |
| $H_o$: $M_{T_i} \neq f(\text{PEAK\_})$ | $F_{1,16} = 0.2513$ | 0.6230 |
| $H_o$: $M_{T_i} \neq f(\text{SEL})$ | $F_{1,16} = 0.2743$ | 0.6077 |

a. Mortal injury vs. PEAK_




b. Mortal injury vs. SEL

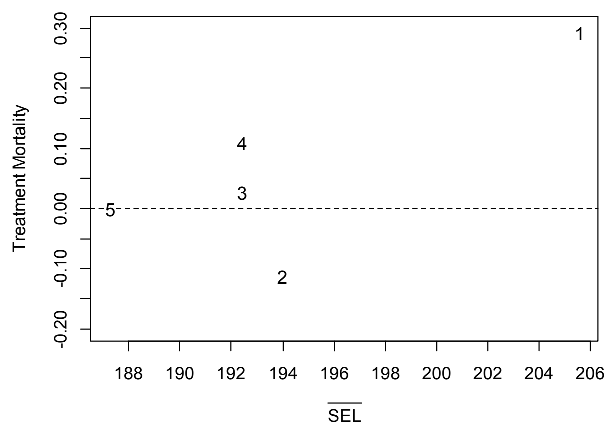



Figure 2. Scatterplots of observed rates of mortal injury after correction for control rates against (a) peak negative sound pressure and (b) sound exposure level. Data were pooled over replicates and exposure levels averaged. Treatments 1–5 are in order of increasing distance from sound source.

## Literature Cited

Finney, D. J. 1971. Probit analysis, Third edition. Cambridge University Press, Cambridge, United Kingdom.

McCullagh, P. and J. A. Nelder. 1989. Generalised linear models, Second edition. Chapman & Hall, London, United Kingdom.

# Attachment A

Table 1. Raw counts of pallid sturgeon with and without mortal injury by test cage and associated levels of measured negative peak pressure (PEAK_) and sound exposure level (SEL) used in data analysis.

| Cage | Alive* | Injured** | Block | Treatment | PEAK_ | SEL |
|---|---|---|---|---|---|---|
| B1D1 | 2 | 1 | 1 | 1 | 224.46 | 205.38 |
| B1D2 | 3 | 0 | 1 | 2 | 222.11 | 199.89 |
| B1D3 | 3 | 0 | 1 | 3 | 211.93 | 191.81 |
| B1D4 | 2 | 1 | 1 | 4 | 210.19 | 190.76 |
| B1D5 | 3 | 0 | 1 | 5 | 205.63 | 186.51 |
| B1DC | 2 | 1 | 1 | 0 | 0 | 0 |
| B2D1 | 3 | 0 | 2 | 1 | 225.38 | 205.75 |
| B2D2 | 2 | 1 | 2 | 2 | 222.26 | 199.88 |
| B2D3 | 2 | 1 | 2 | 3 | 212 | 191.95 |
| B2D4 | 2 | 1 | 2 | 4 | 211.21 | 191.29 |
| B2D5 | 3 | 0 | 2 | 5 | 206.14 | 186.69 |
| B2DC | 3 | 0 | 2 | 0 | 0 | 0 |
| B3D1 | 3 | 0 | 3 | 1 | 224.86 | 205.63 |
| B3D2 | 1 | 1 | 3 | 2 | 222.61 | 200.03 |
| B3D3 | 3 | 0 | 3 | 3 | 213.77 | 192.76 |
| B3D4 | 3 | 0 | 3 | 4 | 211.25 | 191.55 |
| B3D5 | 3 | 0 | 3 | 5 | 205 | 186.01 |
| B3DC | 2 | 0 | 3 | 0 | 0 | 0 |
| B4D2 | 2 | 1 | 4 | 2 | 222.17 | 199.96 |
| B4D3 | 2 | 1 | 4 | 3 | 212.98 | 192.49 |
| B4D4 | 2 | 1 | 4 | 4 | 210.59 | 191.23 |
| B4D5 | 1 | 2 | 4 | 5 | 205.7 | 186.24 |
| B4DC | 1 | 1 | 4 | 0 | 0 | 0 |
| B5D1 | 3 | 0 | 5 | 1 | 225.09 | 205.87 |
| B5D2 | 3 | 0 | 5 | 2 | 222.75 | 200.22 |
| B5D3 | 3 | 0 | 5 | 3 | 212.18 | 192.32 |
| B5D4 | 3 | 0 | 5 | 4 | 210.27 | 191.31 |
| B5D5 | 3 | 0 | 5 | 5 | 205.51 | 186.22 |
| B5DC | 3 | 0 | 5 | 0 | 0 | 0 |

*Alive and without injury
** Mortality or mortal injury

# Attachment B

Table 1. Raw counts of paddle fish with and without mortal injury by test cage and associated levels of measured negative peak pressure (PEAK_) and sound exposure level (SEL) used in data analysis.

| Cage | Alive* | Injured** | Block | Treatment | PEAK_ | SEL |
|---|---|---|---|---|---|---|
| B1D1 | 1 | 3 | 1 | 1 | 222.26 | 204.9 |
| B1D2 | 4 | 0 | 1 | 2 | 214.7 | 193.91 |
| B1D3 | 3 | 0 | 1 | 3 | 211.53 | 192.23 |
| B1D4 | 3 | 0 | 1 | 4 | 210.29 | 192.11 |
| B1D5 | 2 | 0 | 1 | 5 | 206.41 | 186.96 |
| B1DC | 3 | 0 | 1 | 0 | 0 | 0 |
| B2D1 | 3 | 0 | 2 | 1 | 223.73 | 205.77 |
| B2D2 | 3 | 0 | 2 | 2 | 214.25 | 193.68 |
| B2D3 | 2 | 1 | 2 | 3 | 212.71 | 193 |
| B2D4 | 3 | 0 | 2 | 4 | 210.21 | 192.44 |
| B2D5 | 4 | 0 | 2 | 5 | 206.23 | 187.4 |
| B2DC | 3 | 0 | 2 | 0 | 0 | 0 |
| B3D1 | 3 | 1 | 3 | 1 | 223.94 | 206 |
| B3D2 | 4 | 0 | 3 | 2 | 214.57 | 194.32 |
| B3D3 | 2 | 0 | 3 | 3 | 211.63 | 192 |
| B3D4 | 2 | 2 | 3 | 4 | 212.42 | 192.64 |
| B3D5 | 3 | 1 | 3 | 5 | 206.3 | 187.4 |
| B3DC | 3 | 1 | 3 | 0 | 0 | 0 |

*Alive and without injury
** Mortality or mortal injury