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Dear Mr. Theriault,

Hunt Oil Company of Canada, Inc. (HOCC) is pleased to submit a report entitled *Results from the Environmental Effects Monitoring Program of a Seismic Survey in Sydney Bight Conducted in November, 2005* for review.

The report provides the results of the environmental effects monitoring program (EEMP) carried out at the onset of the seismic survey conducted by HOCC in Sydney Bight in November 2005. This report provides the results that focus on the potential for sublethal damage to the sensitive ear structure of fish, in this case Atlantic cod (*Gadus morhua*).

Please do not hesitate to contact me if you have any comments or questions.

Sincerely,

**Hunt Oil Company of Canada, Inc.**

A handwritten signature in blue ink, appearing to read "Paul R. Habenicht".

Paul R. Habenicht  
Vice President & General Manager

# Results from the Environmental Effects Monitoring Program of a Seismic Survey in Sydney Bight Conducted in November, 2005



Prepared for Hunt Oil Company of Canada, Inc.  
Prepared by CEF Consultants Ltd.

November 1, 2006



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## **Acknowledgements**

CEF Consultants Ltd. would like to thank Hunt Oil Company of Canada, Inc. for their support in helping make the EEMP successful. Acoustics technicians and biologists from Oceans Ltd. performed the sound level measurements and dissection and preservation of the fish for shipment. Dr. Arthur N. Popper, Environmental BioAcoustics LLC, Rockville, MD completed the analysis of ear tissues. Thanks also goes to the Nova Scotia Department of Energy for supplying funding to conduct *in situ* video monitoring of the caged cod. DeepSlope Explorations and Connors Diving Services were responsible for design and conduct of this supplemental program. Special recognition is extended to the fishing vessel captains who participated in the program to support improving knowledge about the effects of seismic airguns.

# **Results from the Environmental Effects Monitoring Program of a Seismic Survey in Sydney Bight Conducted in November, 2005**

Prepared for Hunt Oil Company of Canada, Inc.

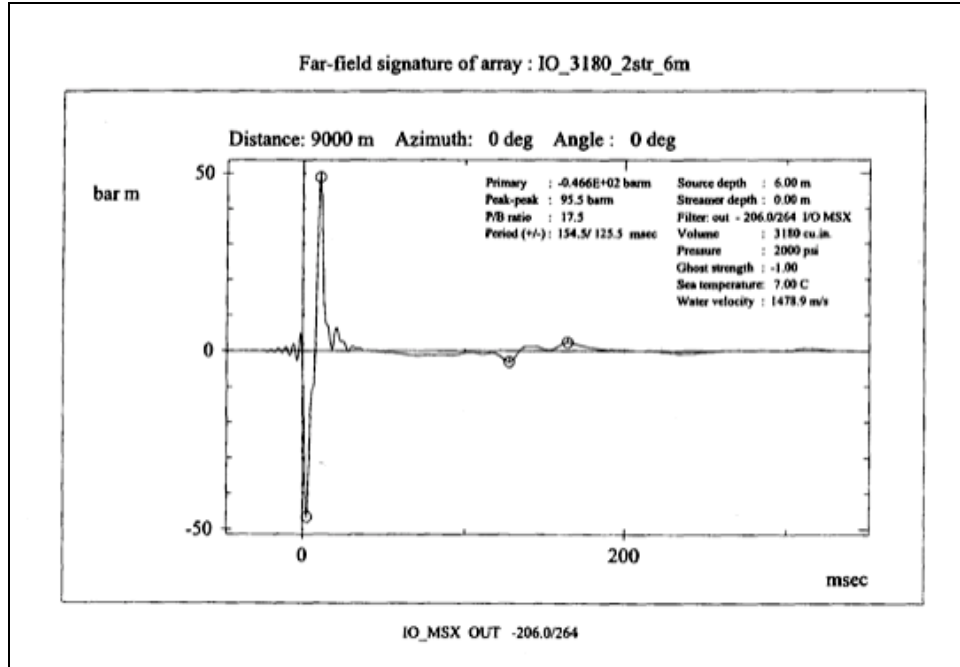
November 1, 2006

## **Introduction**

This report provides the results of the environmental effects monitoring program (EEMP) carried out at the onset of the seismic survey conducted by Hunt Oil Company of Canada, Inc. in Sydney Bight in November of 2005. A final technical report providing a more detailed description of the methodology for various aspects of the program will be completed before the end of 2006. An interim report, which reported on field results such as sound pressure levels observed at test cage locations and test and control fish mortality, was submitted to the Canada-Nova Scotia Offshore Petroleum Board (CNSOPB) in late 2005. This report provides the results that focus on the potential for sublethal damage to the sensitive ear structure of fish, in this case Atlantic cod (*Gadus morhua*).

## **Methods**

The environmental effects monitoring program (EEMP) proceeded on schedule November 1, 2005. The seismic survey vessel, Gulf Pacific, passed within 138 m of the closest test vessel and suspended fish cage with the airguns firing at full pressure. The airgun array was designed to produce a peak to peak sound pressure level of approximately 259 dB re 1  $\mu$ Pa at 1 m. Figure 1 provides the far field signature of the Gulf Pacific array.



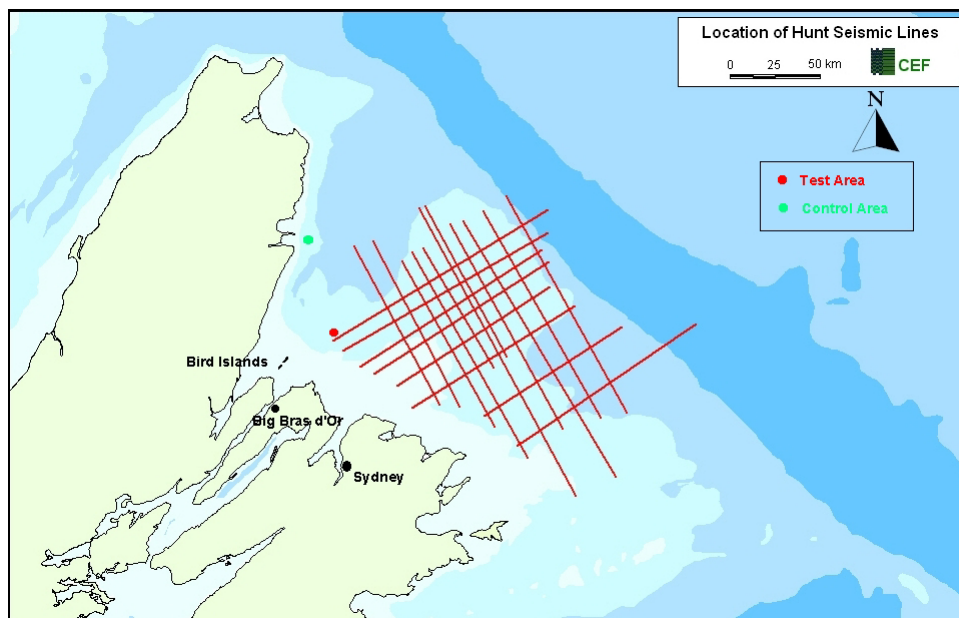
**Figure 1: Far Field Signature of the Gulf Pacific Array**

Approximately 15 hatchery-reared cod were placed in each of seven cages lined with ½-inch mesh (Photo 1). The fish were obtained from GreatBay Aquaculture LLC, 153 Gosling Road, Portsmouth, New Hampshire, and shipped by air from Logan Airport in Boston to the Halifax International Airport in plastic bags filled with oxygen-saturated water inside insulated boxes. From Halifax, fish were transported by truck to the New Campbellton wharf, which acted as the staging area for the program. No fish died during transport and all fish appeared to be in excellent condition at the start of the program.



**Photo 1: Fine Mesh Netting Was Installed Inside A Steel Framed Rectangular Crab Trap**

Two cages were placed at a control site well removed (about 25 km) from any influence of the seismic airgun discharges. Five other cages were placed at a test site for exposure to airgun discharges. Hydrophones to measure actual sound pressure levels were attached to cages at the 100 m and 500 m test locations. The locations of the test and control sites are shown in Figure 2.



**Figure 2: Location of Test and Control Sites for the Hunt EEMP**

Previous studies on the effect of seismic airgun discharges had identified a concern about sublethal damage to delicate ear structures in fish (McCauley et al. 2000), but the distance from the airguns at which this damage occurred was unclear. The primary objective of this EEMP was to identify the threshold of exposure at which minor damage to ear structures occurred in juvenile cod between 10 and 15 cm in total length to help clarify the question of at what distance these types of sublethal impacts occur. Live fish were thus dissected and appropriate ear tissues removed and preserved for later analysis in a laboratory at the University of Maryland. At the laboratory, the tissue was dissected, prepared for examination using Scanning Electron Microscopy and then analyzed for potential damage. Final tissue analysis was completed by Dr. Arthur N. Popper, Environmental BioAcoustics LLC, 14114 Parkvale Road Rockville, Maryland.

Additional information on the effect of airgun discharges was obtained using video cameras observing fish behaviour in the cages during exposure. This study was a separate component funded by the NS Department of Energy. Preliminary analysis of the video indicated fish response to airgun discharges was minimal, with only a small startle response when the airguns were at their closest.

The experimental design placed cages at 100 m, 500 m and 1000 m from the path of the seismic survey vessel. In the field these distances could only be approximated, but the distance labels are used to identify the various groups of fish used in the EEMP. Table 1 indicates the numbers of fish and groups in the experimental design.

**Table 1: Number of Fish by Test Group and Control in Experimental Design**

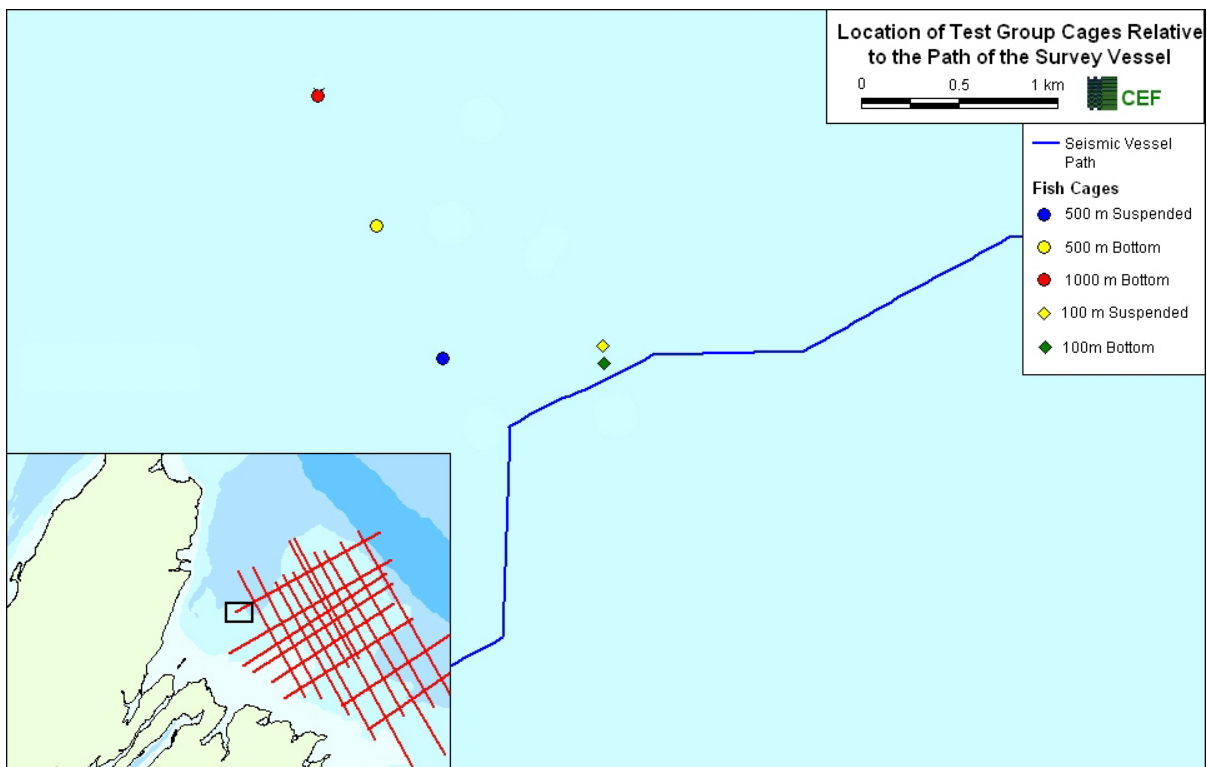
Fish Group	Nominal Distance from Survey Line			Control/Reference
	100 m	500 m	1000 m	
Reference				5
After 5 hour exposure	10	10	10	10
After 5 day exposure	10	10		10
Total # of Fish	20	20	10	25
Total # of Groups	2	2	1	3

In preparation for arrival of the seismic survey vessel, one cage was placed on the sea bottom at nominal distances of 100 m, 500 m, 1000 m and control sites in water depth of approximately 120 m. Depth was greater than anticipated and thus cages with camera or hydrophones attached needed to be suspended from the fishing vessel to allow cables to reach the recording equipment onboard. This meant additional cages, intended to be placed on the bottom at 100 m, 500 m and control sites, were suspended from fishing vessels. In all cases, control fish were treated as similarly to test fish as possible.

Problems were encountered with positioning of the fishing vessels carrying the fish cages due to:

- water depth made anchoring difficult;
- sea conditions were rough and windy making the vessel swing from its anchor line, and
- one vessel had a Loran positioning system, whereas the other two vessels had more accurate GPS systems.

Nonetheless, the positions of the various traps reasonably approximated the experimental design. A comparison of the experimental design and actual distances from the path of the seismic survey vessel are provided in Table 2. Figure 3 shows the local of the cages relative to the path of the seismic survey vessel.



**Figure 3: Location of Test Group Cages Relative to the Path of the Survey Vessel**

**Table 2: Comparison of Experimental Design and Actual Cage Distances from Survey Vessel Path**

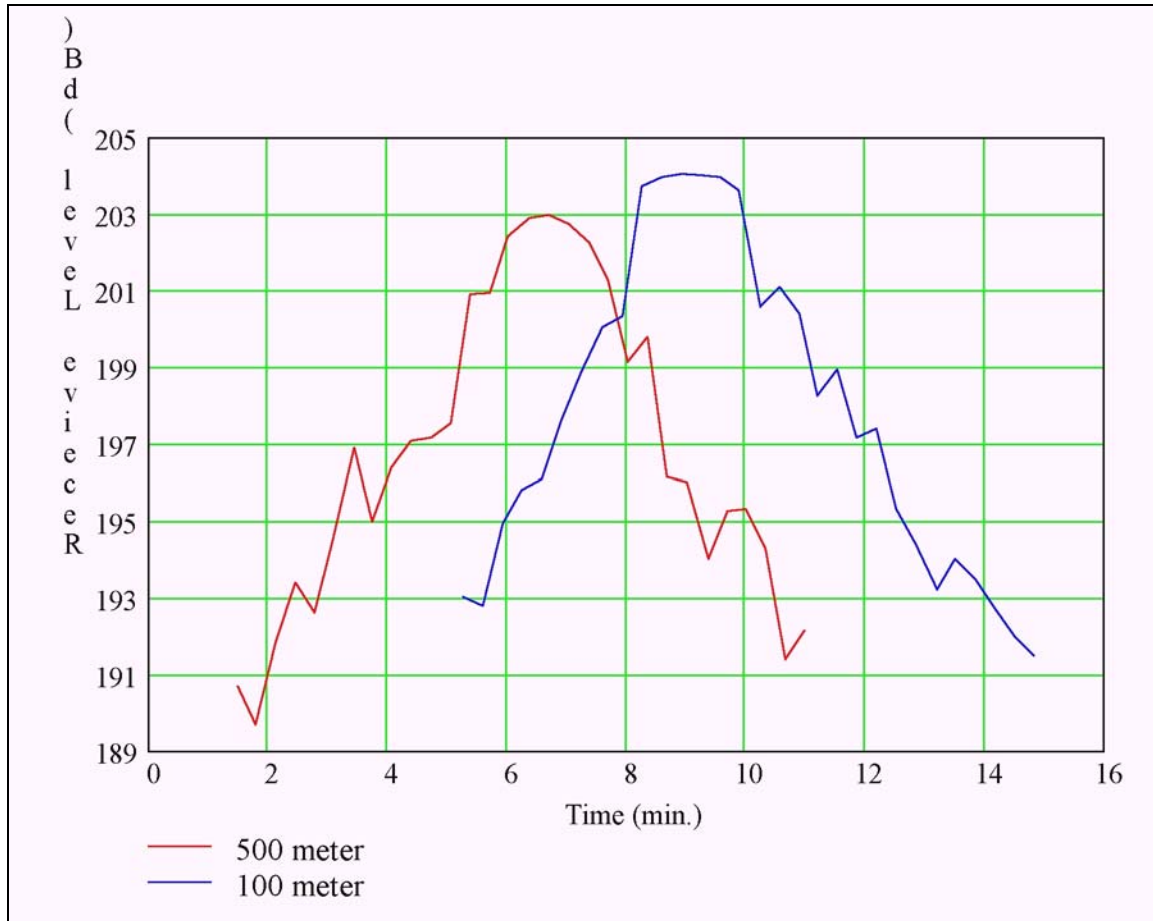
<b>Experimental Design Distance</b>	<b>Actual Distance from Seismic Survey Vessel</b>
100 m Suspended Cage	138 m*
100 m on Bottom Cage (5 day recovery)	55 m
500 m Suspended Cage	340 m
500 m on Bottom Cage (5 day recovery)	860 m
1000 m on Bottom Cage	1360 m

\*Distance measured with laser distance finder

The two test and one control suspended cages were recovered as soon as the seismic survey vessel had proceeded more than 500 m from the test sites. The cage on the sea bottom at the 1000 m site was also recovered as soon as possible after the survey vessel had passed.

## Results

Results from the hydrophone recording of sound pressure levels are provided in Figure 4. The sound source level was 259 dB re 1  $\mu$ Pa at 1 m and maximum exposure levels were 204 dB<sub>(p-p)</sub> re 1  $\mu$ Pa at 1 m.



**Figure 4: Recorded Sound Pressure Levels at Suspended Fish Cages**

Mortality from immediate recovery of the first four cages was 22%, most likely due to environmental conditions, notably low temperature (about 2.4°C), high tidal currents, and deep water (120 m). In cases recovered immediately, control mortality was 30.8%, and average mortality in the three cages near the path of the seismic survey vessel was 19.6%. Control and test mortality is considered to have occurred as a result of stress induced by the handling, exposure of the fish to large temperature fluctuations and erratic currents within the cages.

Three cages were left for retrieval for 5 days following the test exposure to check for injuries that took time to appear. Two of these cages were retrieved on Sunday, November 6. Weather had prevented recovery of the cages earlier. A third cage at the 500 m site could not be located and has been abandoned. Results from all cages are summarized in Table 3.

**Table 3: Condition of Fish and % Mortality in Test and Control Cages**

Fish Condition	100 m	100 m after 5 days	500 m	1000 m	Control	Control after 5 days
Dead before Test	0	0	0	0	4	0
Missing	0	9	0	0	0	9
Dead	0	0	2	2	4	1
Dying	1	3	3	0	0	0
Unlively	8	1	0	1	0	2
Live	5	2	10	8	9	1
% Mortality	7.1%	80%	33.3%	18.2%	30.8%	76.9%

The delayed recovery of fish demonstrates the harsh conditions in the cages on the sea bottom. One fish in the initial test group was reduced to a bare skeleton within the 5 hours on the bottom. The rapid disintegration of this fish suggests seabed organisms, likely a form of mud flea, were present and a threat to fish on the bottom of the cages. The control cage started with 13 fish, but only 4 were recovered, whereas the test cages started with 15 fish and only 6 were recovered. These fish are assumed to have died and have been totally consumed by mud fleas within the cages when they were in contact with the bottom muds. There were no openings in the fine mesh on the cages that would have allowed a fish to escape. If all fish initially in the cages are considered dead and used in calculating mortality, control mortality becomes 76.9% and test mortality 80.0%.

Even though mortalities averaged 22% in cages recovered immediately after exposure and about 78% in delayed recovery cages, sufficient healthy fish were available to allow statistically acceptable analysis of possible ear tissue injury. The mortalities observed do not suggest lethal impacts resulted from exposure to the airguns at distances as close as 55 m.

### Histology

The histological analysis was carried out at the Atlantic Veterinary College in Prince Edward Island to screen for significant diseases and or morphological changes in the cod fish, particularly in organs.

Sixteen fish were included in the histological analysis. Two fish were sampled from Group A (reference group). Eight cod from Group B (sampled 5 - 6 hours after testing) and six fish from Group C (sampled five days after testing) were analyzed. The histological analysis was completed by Dr. David Groman, Fish Pathologist, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI.

Overall, the fish did not show any significant morphological changes. However, four of the cod were harboring a microsporean infection (*Loma* sp.). Three of the fish were from Group B (one 500 m test group fish and two 1000 m group fish) and one fish was from Group C

(control group). The parasite *Loma* is common in cod, both wild and farmed where a flow through seawater system is employed. Another common finding was the mild to moderate overgrowth of the intestinal lumen by bacterial flora. All the cod with the bacterial flora overgrowth belonged to Group C with fish from the control and 100 m groups.

Ear Tissue

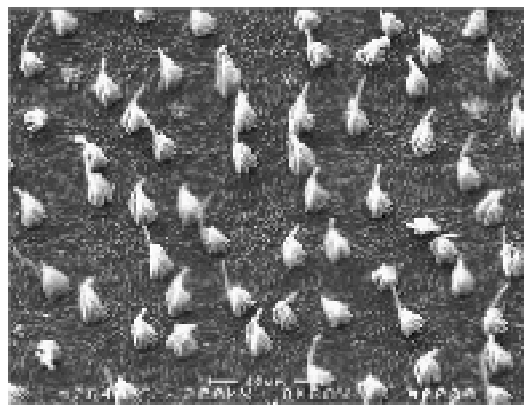
Electron Microscopy was used to examine the effects on the auditory sensory epithelia of Atlantic cod (*Gadus morhua*) of exposure to seismic airguns. Preserved tissue surrounding the ear was shipped to the laboratory in Maryland and the tissue was subsequently dissected, prepared for examination using Scanning Electron Microscopy (SEM) and then analyzed for potential damage. Table 4 identifies the test groups from which fish ears were examined.

**Table 4: Number of Fish by Test Group and Control Analyzed**

Fish Group	Nominal Distance from Survey Line			Control/Reference
	100 m	500 m	1000 m	
Reference				2
After 5 hour exposure	2	2	--	3
After 5 day exposure	1	**		1
Total # of Fish	3	2	0	6
Total # of Groups	2	1**	0	3

\*\*indicates that this trap was not recovered following the 5 day exposure thus no samples could be submitted.

Tissue was examined in animals from different test groups and from controls. Much of the tissue could be viewed with SEM. However, some tissue artifacts were found that can be attributed to tissue preparation. This is supported by finding the same abnormalities in control (unexposed) and exposed animals. Photo 2 illustrates right saccule tissue from the ear of a typical test fish.



**Photo 2: Right Saccule from a Fish Exposed to Seismic Survey Airguns**

This is a right sacculle and shows ciliary bundles in good condition on the sensory cells. Ciliary bundles in different otolithic end organs, or even on different regions of the same end organ, may vary in size and shape. The functional significance of this is not yet understood.

Based on preliminary analysis, there does not, at this point, appear to be damage that can be attributed to exposure of fish to sound. Additional analysis is being conducted with more tissue and a final analysis will be provided.

## Conclusions

Based on preliminary analysis, juvenile cod exposed to a typical seismic air gun array at distances as close as 55 m to the survey vessel in cages at depths of 120 m did not show signs of damage to sensitive ear tissue when examined under Scanning Electron Microscopy. Mortality in test and control groups was moderately high but very similar because of exposure to deep cold water and strong currents. Gross histological examination did not indicate any physical affects on organs or other tissues. Video recording of the caged fish showed constant upward swimming motion that was only slightly affected by even the closest exposure to the airguns.

## REFERENCES

- McCauley, R.D., J. Fewtrell, A.J. Duncan, C. Jenner, M.-N. Jenner, J.D. Penrose, R.I.T. Prince, A. Adhitya, J. Murdoch and K. McCabe. 2000. Marine seismic surveys: Analysis of and propagation of air-gun signals; and effects of air-gun exposure on humpback whales, sea turtles, fishes and squid. Prepared for Australian Petroleum Production and Exploration Association. Centre for Marine Science and Technology, Curtin University. Perth. Australia. 198p.
- Popper, Arthur N. 2006. Effects of Exposure to Seismic Air-Guns on the Ears of Atlantic Cod (*Gadus morhua*). Interim Report prepared for Hunt Oil Company of Canada, Inc., Calgary, AB, September 3, 2006: 5p.

**Appendix 1. Cod Ear Analysis**

EEMP for Seismic Survey in Sydney Bight

**Cod Ear Analysis**

Date: November 2005

Fish #	Date	Dissection time	Group*	Length (cm)	Weight (g)	Cod ear in Karnovsky's	External Visible Abnormalities	Photo #	Other
1	1-Nov	8:00	A	12.5	16	Y	None		
2	1-Nov	8:24	A	14.5	24	Y	None		
3	1-Nov	8:45	A	14.1	23	Y	None		
6	1-Nov	21:46	B-100	12.3	13	Y	None		
7	1-Nov	22:13	B-Control	13.4	20	Y	None		Not lively
8	1-Nov	22:27	B-500	14.1	22	Y	None		
9	1-Nov	22:41	B-1000	12.6	16	Y	None		
10	1-Nov	22:54	B-Control	11.6	14	Y	None		Not lively
11	1-Nov	23:09	B-100	15.5	31	Y	None		
12	1-Nov	23:22	B-500	14.5	26	Y	None		
13	1-Nov	23:33	B-1000	14.5	25	Y	None		
14	1-Nov	23:50	B-500	13.6	20	Y	None		Not lively
15	2-Nov	0:04	B-Control	13.4	17	Y	None		
16	2-Nov	0:15	B-100	13.5	19	Y	None		
17	2-Nov	0:25	B-1000	14.6	23	Y	None		
18	2-Nov	0:36	B-500	13.1	19	Y	None		
19	2-Nov	0:46	B-Control	13.3	19	Y	None		
20	2-Nov	0:54	B-100	12.7	14	Y	None		

21	2-Nov	1:04	B-1000	13	19	Y	None		
22	2-Nov	1:14	B-500	14.2	23	Y	None		
23	2-Nov	1:23	B-Control	15	29	Y	None		
24	2-Nov	1:32	B-100	13.9	19	Y	None		
25	2-Nov	1:42	B-1000	17.6	34	Y	None		Fish dead
26	2-Nov	1:52	B-500	13.8	20	Y	None		Fish dead
27	2-Nov	2:03	B-Control	12	13	Y	None		Fish dead
28	2-Nov	2:11	B-100	13.7	19	Y	None		
29	2-Nov	2:21	B-1000	14.7	23	Y	None		Fish dead
30	2-Nov	2:31	B-100	13.6	19	Y	None		
31	2-Nov	2:42	B-500	14.8	26	Y	None		Fish dead
32	2-Nov	2:52	B-Control	13	17	Y	None		Fish dead
33	2-Nov	3:01	B-1000	13.7	20	Y	None		Fish dead
34	2-Nov	3:08	B-100	11.9	14	Y	None		Fish dead
35	2-Nov	3:19	B-500	12.3	15	Y	None		Fish dead
36	2-Nov	3:27	B-Control	11.4	12	Y	None		Fish dead
37	2-Nov	3:35	B-1000	14.8	25	Y	None		Fish dead
46	6-Nov	16:03	C-Control	14.7	23	Y	None		
47	6-Nov	16:17	C-Control	14.2	22	Y	Red fins		
48	6-Nov	16:28	C-100	15.1	25	Y	None		
49	6-Nov	16:40	C-100	15.4	26	Y	None		
50	6-Nov	16:52	C-Control	14.5	25	Y	Red fins		Fish dead (upon arrival)
51	6-Nov	17:03	C-100	13.5	21	Y	None		Fish dead (upon arrival)
52	6-Nov	17:16	C-Control	14.2	26	Y	None		Fish dead (upon arrival)
53	6-Nov	17:23	C-100	14	21	Y	None		Fish dead (upon arrival)
54	6-Nov	17:30	C-100	14.9	25	Y	None		Fish dead (upon arrival)
55	6-Nov	17:39	C-100	12.5	18	Y	None		Fish dead (upon arrival)

\* Group A: Reference (3 fish)

Group B: ~ 5-6 hours after testing 100m (8 fish),  
 B-Con = Control fish (8 fish), B-100 = Exposed fish @ 1000m (8 fish)  
 B-500 = Exposed fish @ 500m (8 fish), B-1000 = Exposed fish @ 1000m (8 fish)

Group C: 5 days after testing  
 C-Con = Control fish (4 fish), C-100 (6 fish)

**Appendix 2. Histological Analysis**

**EEMP for Seismic Survey in Sydney Bight  
(November 2005)**

**Histological Analysis**

**Date: November 2005**

Fish #	Date	Dissection time	Group*	Length (cm)	Weight (g)	Head in Formalin	Gill in Formalin	Internal organs in Formalin	External Visible Abnormalities	Photo #	Other
4	1-Nov	10:00	A	14.9	26	x		x	0	0	
5	1-Nov	10:10	A	13.1	17	x		x	0	0	
38	2-Nov	3:52	B-100	13	17	x		x	0	0	Fish dead
39	2-Nov	3:56	B-100	13.5	19	x		x	0	0	Fish dead
40	2-Nov	4:04	B-500	13	17	x		x	0	0	Fish dead
41	2-Nov	4:09	B-500	13.9	20	x		x	0	0	Fish dead
42	2-Nov	4:16	B-1000	13.1	17	x		x	0	0	Fish dead
43	2-Nov	4:20	B-1000	12	14	x		x	0	0	Fish dead
44	2-Nov	4:28	B-Control	13.9	22	x		x	0	0	Fish dead
45	2-Nov	4:33	B-Control	14.3	26	x		x	0	0	Fish dead
46	6-Nov	16:03	C-Control	14.7	23		x	x	0	0	
47	6-Nov	16:17	C-Control	14.2	22		x	x	Red fins	0	
48	6-Nov	16:28	C-100	15.1	25		x	x	0	0	
49	6-Nov	16:40	C-100	15.4	26		x	x	0	0	
50	6-Nov	16:52	C-Control	14.5	25		x	x	Red fins	0	Fish dead (upon arrival)

51	6-Nov	17:03	C-100	13.5	21		x	x	0	Fish dead (upon arrival)
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\* Group A: Reference (2 fish)

Group B: ~ 5-6 hours after testing B-Con = Control fish (2 fish), B-100 = Exposed fish @ 100m (2 fish),

B-500 = Exposed fish @ 500m (2 fish), B-1000 = Exposed fish @ 1000m (2 fish)

Group C: 5 days after testing

C-Con = Control fish (3 fish), C-100 (3 fish)