Effects of exposure to seismic airgun use on hearing of three fish species

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Seismic airguns produce considerable amounts of acoustic energy that have the potential to affect marine life. This study investigates the effects of exposure to a 730 in.^{3} airgun array on hearing of three fish species in the Mackenzie River Delta, the northern pike (*Esox lucius*), broad whitefish (*Coregonus nasus*), and lake chub (*Couesius plumbeus*). Fish were placed in cages in the 1.9 m of water and exposed to five 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 using the auditory brainstem response (ABR). Threshold shifts were found for exposed fish as compared to controls in the northern pike and lake chub, with recovery within 24 hours of exposure, while there was no threshold shift in the broad whitefish. It is concluded that these three species are not likely to be substantially impacted by exposure to an airgun array used in a river seismic survey. Care must be taken, however, in extrapolation to other species and to fishes exposed to airguns in deeper water or where the animals are exposed to a larger number of airgun shots over a longer period of time.
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INTRODUCTION

There is growing interest in the effects of anthropogenic (human-generated) sounds on marine mammals (e.g., Myrberg, 1980; Richardson *et al.*, 1995; NRC, 2003, 2005; Wartzog *et al.*, 2004) and fishes (Popper, 2003; Popper *et al.*, 2004). The continuum of potential effects on such animals could range from immediate death to no response whatsoever. In between are a range of effects that may include damage to various body tissues that could impair or ultimately kill the animal, temporary or permanent hearing threshold shift, changes in behavior because animals try to avoid the sound, and behavioral effects resulting from an animal not being able to hear biologically important environmental sounds or communication sounds from conspecifics (e.g., Richardson *et al.*, 1995; Popper, 2003; Popper *et al.*, 2004; Wartzog *et al.*, 2004).

A wide range of anthropogenic sound sources are present in the marine and freshwater environments including shipping, high power sonar, and echo sounders. Ambient levels resulting from such sounds appear to be increasing. However, it is very difficult to quantify this increase due to the diversity of sound sources in the marine environment and the very limited data available on underwater sounds in most parts of the world (NRC, 2003; Wartzog *et al.*, 2004).

Airguns are widely used for marine-based seismic exploration by the oil and gas industry. These devices produce a compressed air bubble that collapses under the pressure of water causing a sharp concussive ‘‘explosion.’’ The peak sound levels of individual airguns are as high as 230 dB (re 1μPa) at a range of 1 meter from the source. Arrays of airguns are trailed behind a vessel and put out frequent ‘‘shots.’’ The sounds reflect off geologic formations below the water

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bottom and are detected by long streams of hydrophones towed behind the vessel. By measuring time of arrival and other characteristics of the reflected signal, it is possible to predict the presence of oil or gas in the sea bottom.

Despite the increasing interest of scientists, regulators, and environmental groups in anthropogenic sounds, there are very few experimental data that directly address how these sources affect animals. Data for fishes show that exposure to moderately loud noises can result in temporary hearing loss (called Temporary Threshold Shift, TTS) in a few species that have been studied including goldfish (*Carassius auratus*) and other fishes specialized for hearing (Popper and Clarke, 1976; Scholik and Yan, 2001, 2002; Amoser and Ladich, 2003; Amoser *et al.*, 2004; Smith *et al.*, 2004a, 2004b) (see Popper and Carlson, 1998 and Popper *et al.*, 2003 for reviews of fish hearing). Three studies using higher intensity sounds have also shown damage to the sensory hair cells of the inner ear, the cells responsible for transducing sound into neural impulses (Enger, 1981; Hastings *et al.*, 1996; McCauley *et al.*, 2003).

In the only published study to examine the direct effects of an airgun on fish physiology, McCauley *et al.* (2003) determined the effects of exposure to an airgun on the structure of fish ears. They found that exposure to multiple shots from an airgun over several hours produced damage to the sensory epithelia of the saccule, the major auditory end organ of the ear, in a group of caged pink snapper (*Pagrus auratus*), an Australian marine fish. Evidence for damage showed up as early as 18 hours post-exposure and was very extensive when fish were examined 58 days post-exposure as compared to controls.

McCauley *et al.* (2003) did not, however, test whether there were any effects on fish hearing. Indeed, the effect of anthropogenic sources on hearing is an important question since it is possible to have TTS without any permanent effects on the structure of the ear. Such TTS has the potential to put a fish in danger, since it may not hear the sounds of predators, mates, or the environment.

In the current study we examined the effects of exposure to a seismic airgun array on three species of fish found in the Mackenzie River Delta near Inuvik, Northwest Territories, Canada, an area in which there have been extensive land-based and marine seismic surveys to map rich gas and oil reserves. Considerable concern has arisen among the local population and regulators regarding proposed riverine seismic surveys and whether the sounds from the airguns could negatively impact fisheries resources (Cott *et al.*, 2003). The three species chosen for study not only represent the diversity of the fish fauna of the Mackenzie Delta region, but they also represent diversity in hearing structures found among fishes.

The species included a hearing specialist, the lake chub (*Couesius plumbeus*), and two fishes without known hearing specializations, the northern pike (*Esox lucius*), and a coregonid, the broad whitefish (*Coregonus nasus*). Broad whitefish were selected because they are one of the most important species to the aboriginal subsistence fisheries in the lower Mackenzie River and Delta (Tallman and Reist, 1997).

Fish were exposed to shots from a 730 $in.^3$ airgun array using a paradigm that would produce an exposure comparable to a worst case scenario that a fish would experience from a seismic survey in a river. The fish were tested post-exposure to measure TTS in comparison to control fish, and whether there was recovery from any TTS that was found.

METHODS

Work was done at the Mackenzie River Delta using the Fisheries and Oceans Canada facilities in Inuvik, NT. Lake chub and young of the year (YOY) northern pike were collected using beach seines along the river bank in water depths up to 1.5 m, while broad whitefish and adult northern pike were captured with short set monofilament gill nets (3.81 cm [1.5 in.]–13.97 cm [5.5 in.]) in 1.0–3.0 m of water. The northern pike were held in large tanks with flowing river water fed with external pumps at the experimental site until used. The more sensitive broad whitefish were contained in a pen located within the river itself until the airgun array was in position and then placed in a large tank on shore with flowing river water. Lake chub and YOY pike were held separately in smaller tanks of temperature controlled river water equipped with aeration and filtration in the DFO facility. All animals not used in experiments within 96 hours of capture were released. This study was approved by the Fisheries and Oceans Canada Animal Care Committee.

Measures of hearing

Hearing capabilities were measured using the auditory brainstem response (ABR), a noninvasive method of measuring the whole brain response to auditory stimuli (Corwin *et al.*, 1982; Kenyon *et al.*, 1998; Mann *et al.*, 2001; Scholik and Yan 2001, 2002; Smith *et al.*, 2004a, 2004b). ABR allows for a rapid assessment of hearing (15–20 minutes per fish) without training, and so it is possible to ascertain hearing loss very soon after exposure to sound.

Experiments were conducted with fish restrained in a mesh sling and suspended underwater in a large plastic cooler containing aerated river water (aeration was not used during testing). Lake chub and YOY northern pike were suspended so that the top of the head was approximately 9 cm below the surface of the water and 40 cm away from the underwater speaker (Aqua Synthesis). Because of their large size (350–670 mm), adult northern pike and broad whitefish were suspended so that they were at the same depth but 30 cm from the speaker. Water temperature ranged from 18 °C–20 °C.

A stainless steel recording electrode (Rochester Electro-Medical Inc., Tampa, FL) was inserted subdermally into the medial dorsal surface of the head over the brainstem while a similar reference electrode was placed into the dorsal midline surface of the fish near the anterior insertion of the dorsal fin. A ground electrode was placed in the water near the body of the fish. All exposed surfaces of the electrode tips that were not in direct contact with the fish were coated with enamel for insulation.

Sound stimuli were presented and ABR waveforms collected using a Tucker-Davis Technologies (TDT) physiology apparatus using SigGen and BioSig software (Tucker-Davis Technologies Inc., Gainesville, FL). Sounds were computergenerated using TDT software and passed through a power amplifier (Hafler P1000) connected to the underwater speaker (Aqua Synthesis). Tone bursts were 50 ms in total duration and were gated with a Hanning window (similar to the conditions of past ABR studies in our laboratory; e.g., Mann *et al.*, 2001; Higgs *et al.*, 2001). Responses to each tone burst at each SPL were collected using the BioSig software package, with up to 1000 responses averaged for each stimulus frequency and level combination. In order to speed testing, if an evoked potential was obvious before 1000 averages was reached, the program was advanced to the next test condition. Sounds were presented 17.5 times per second. The SPLs of each presented frequency were confirmed using a calibrated underwater hydrophone (calibration sensitivity, −212 dB re 1 V/μPa; Reson TC 4013; 1 Hz–170 kHz response).

Sound intensity at each frequency was decreased in 6 dB steps until a stereotypical ABR was seen and then advanced to the next lower level (Fig. 1). Threshold was defined as the lowest level at which a response could be seen in the Fourier transform of the evoked potential that was 3 dB above background noise.

ABRs were determined for experimental, control, and baseline animals. Baseline animals were from the collected group but they were not placed in the experimental pen, and they thus served as controls for handling. The control group consisted of animals that were placed into the experimental pen and lowered to experimental depth and kept there for a period of time equivalent to the insonification period of experimental animals but without the sound exposure. Control and baseline results were similar, but baseline data are not reported here since they will be presented in a comparative study of hearing in a wide range of Mackenzie Delta species (Mann *et al.*, unpublished).

Once fish were tested with ABR they were deeply anesthetized with buffered MS-222 (an anesthetic for cold-blooded vertebrates) and then weighed and measured. The fish were then prepared for electron microscopic analysis to determine any effects on inner ear tissues (Popper *et al.*, unpublished).

Exposure paradigm

Broad whitefish and adult northern pike were placed into a 1 cubic meter holding pen made of 6.4 mm (1/4 inch) seine netting on a frame made of 12.8 mm (1/2 inch) metal rebar (top and bottom) and 12.8 mm (1/2 inch) lead-line (sides). The top of the pen had a hinged and latched wooden lid to allow access to the fish. The cage was set at the desired depth using floatation above and anchors below at the end of a fixed dock. Lake chub and YOY pike were exposed by placing them in a galvanized Gee minnow trap, the entrances to which were sealed with plastic netting. The experiments were conducted in 1.9 m of water with the pen and trap submerged so that they were centered about 1 m below the surface. Different fish species and life stages were exposed separately. For each test the fish were placed in the river and the airgun array was fired either five or 20 times.

Following sound exposure, the pen was lifted so that the top was just above the surface of the water. The fish were captured with a wetted net and placed into a holding tank with fresh river water. They were then taken by truck to the Fisheries and Oceans Canada lab (about a 90 second drive) where they were placed into aerated and filtered holding tanks of temperature controlled river water until they were used for ABR. With the exception of fish that were intentionally held for up to 24 hours post-exposure to look at recovery, all fish had ABRs measured within 1.5 hours of airgun exposure.

Seismic airgun array

The airgun array was a clustered array of eight equally spaced (70 cm between guns) SGI and SGII type sleeve guns with a total volume of 730 in.^{3} (12,000 cc) and with a total array dimension of 2.6 m in length and 1.22 m across. The volume of individual guns in the array ranged from 70 in.^{3} (1150 cc) to 150 in.^{3} (2460 cc). The airguns were deployed at 1.8 meters depth, were charged from a single air compressor and were fired with approximately 1900 psi (13.1 kPa) chamber pressure. The airgun array was fired manually and this led to small variations in the firing pressure of the airguns. The observed variability in the received level at the fish cages (see Table I) may be attributed to the manual firing of the airgun array.

The airgun array broadside was pointed toward the fish cage so that its maximum lateral pressure was radiated in the direction of the cage. The airgun array was positioned so that the cages were in the far field of the signal (i.e., where the pressure wave forms from the airgun added constructively). The position of the airgun array shifted slightly during 28 July, the first day of testing, due to high winds at the study site and had to be repositioned on 29 July, the second day of testing. The airgun array was 17 m from the fish cages on the first day of testing and 13 m on the second day of testing.

Airgun calibration

Both acoustic pressure and the acoustic particle velocity were measured directly adjacent to the fish cage during the exposure tests. Received levels inside the cage were not expected to be significantly different than those measured outside the cage. Measurements performed by JASCO Research Ltd. for a prior study, using similar mesh cages, indicated that airgun levels were not measurably different inside and outside of the cages (MacGillivray *et al.*, 2002).

The particle velocity was computed from differential measurements of the acoustic pressure using the pressure gradient method (Fahy, 1977). To do this, JASCO Research Ltd. designed and built an apparatus that consisted of a copper frame constructed so as to describe the three perpendicular Cartesian axes (x, y, and z). The distance from the origin point to each of the axis-ends was 50 cm. Four calibrated Reson TC4043 hydrophones (nominal sensitivity −201 dB re V/μPa) were mounted at the axis-ends while a single calibrated Reson TC4034 reference hydrophone (nominal sensitivity −218 dB re 1 V/μPa) was placed midway along the z axis of the pressure gradient sensor. A JASCO Research Ltd. UWINSTRU depth/attitude monitoring sensor mounted

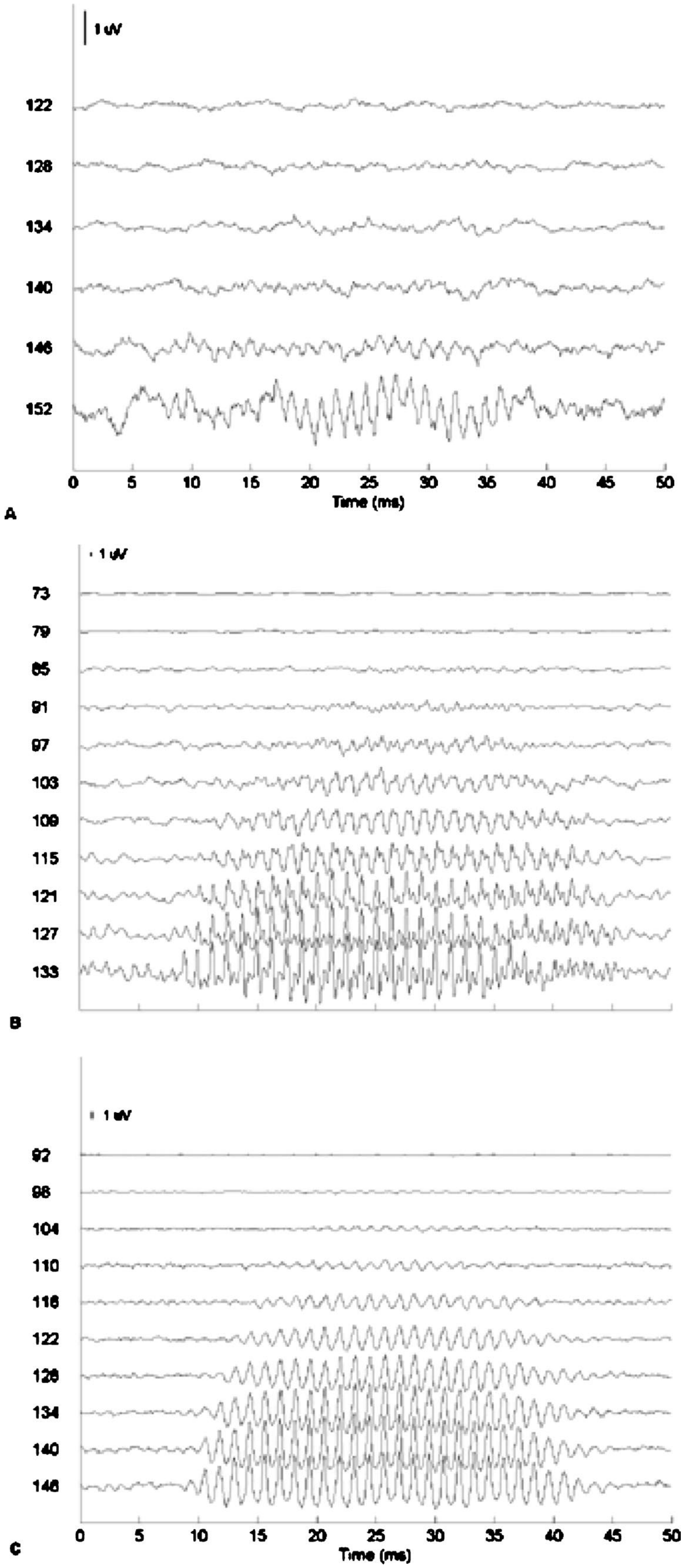

FIG. 1. Representative auditory brainstem response traces for a 400 Hz sound. The sound level (dB re 1 μPa) used for each trace is shown on the y-axis. The amplitude of the evoked potential is indicated by 1 μV bar shown at the top of each plot. Note that the scale is different for each plot. (A) Broad whitefish, (B) lake chub, and (C) adult northern pike.

along the x-axis of the frame to establish orientation of the apparatus for calculations of pressure and particle displacement.

The signals from the five hydrophones and the UWINSTRU were fed through custom shielded underwater cables to a laptop based acquisition system. The hydrophone signals were digitized using a Quatech DAQP-16 PCMCIA acquisition card using custom software and recorded to hard

TABLE I. Calibration data for each airgun shot and mean data for all shots. Tests 1–5 were on 7/28/2004. Tests 6–10 were on 7/29/2004. The various metrics used are discussed in the text.

Test	Species	Mean peak SPL (dB re 1 μPa)	Mean 90% RMS SPL (dB re 1 μPa)	Mean SEL (dB re 1 $\mu Pa^2 \cdot s$)	Mean peak velocity level (dB re 1 nm/s)	Mean 1 s RMS velocity level (dB re nm/s)	Mean peak intensity level (dB re 1 μPa)	Mean 1 s RMS intensity level (dB re 1 μPa)
1	Broad whitefish	209.5	199.4	180	139.6	112.6	202.6	176.5
2	Adult northern pike	207.3	197.7	178.3	139	111.7	202.0	175.2
3	Adult northern pike	207.5	198	178.3	139.4	112.1	202.1	175.5
4	Broad whitefish	209.9	199.8	179.7	140.7	113.3	203.4	176.8
5	Lake chub	205.2	195.1	175.9	136.7	109.7	200.1	173.1
6	Lake chub	206.8	197.9	177.9	140.2	112.1	202.4	175.6
7	Lake chub	205.7	196.2	176.7	136.9	110.1	199.5	173.7
8	Lake chub	207.1	197.4	177.5	138.5	111.2	201.0	174.5
9	YOY northern pike	207.5	197	177	139.5	110.4	201.8	173.6
10	YOY northern pike	206.2	195.3	175.9	136.7	108.8	199.1	171.6
Average		207.3	197.4	177.7	138.7	111.2	202.6	176.5
MIN		205.2	195.1	175.9	136.7	108.8	202.0	175.2
MAX		209.9	199.8	180	140.7	113.3	202.1	175.5

disk. The digital sampling rate for the acoustic signals was 20 kHz on each channel with 16-bit resolution (± 10 V maximum range). In addition, the signal from the single TC4034 reference hydrophone was amplified using an Ithaco 451M programmable gain amplifier. The TC4043 hydrophones have built-in preamplifiers and were not amplified prior to digitizing. The orientation and depth measured by the UWINSTRU were communicated via serial interface to the laptop and recorded into the logbook.

Acoustic metrics

For each exposure test, average received sound levels are reported using three standard metrics for periodic transient sources (as described in Richardson *et al.*, 1995): peak sound pressure level (Peak SPL), 90% RMS sound pressure level (90% RMS SPL), and sound exposure level (SEL) (see Table I). Sound pressure levels are reported in dB re 1 μPa and sound exposure levels in dB re $\mu Pa \cdot s^2$. Acoustic pressure was measured in the frequency band 2 Hz–10 kHz.

The average acoustic particle velocity was measured for each exposure and is reported using two metrics, peak velocity level and 1 second RMS velocity level. Particle velocity levels are reported in dB re nm/s (the ANSI standard acoustic reference velocity). The finite baseline of the differential pressure measurement placed an upper limit on the maximum frequency at which particle velocity could accurately be measured. Thus, particle velocity was low-pass filtered at 1325 Hz, which corresponds to the 3 dB point of the estimated error of the velocity measurement. In addition, particle velocity was high-pass filtered at 150 Hz to reject low-frequency noise.

Acoustic intensity was computed from the product of the pressure and velocity traces. For each exposure, intensity is reported using two metrics, peak intensity level and 1 second RMS intensity level. Intensity level measurements are reported in dB re 0.676×10^{-18} W/m^2, the intensity of a 1 μPa plane wave.

Calibration of airgun array

A total of 10 noise exposure tests were performed on 28 and 29 July 2004 (Table I). Figure 2(A) shows a representative pressure waveform and its associated frequency spectrum as measured at the fish cages during the noise exposure tests. Figure 2(B) shows the velocity amplitude trace for the same shot, along with the frequency spectrum of the three axial velocity traces. Calibration data are summarized in Table I for each test. The calibration results for each test did not differ substantially from the average of all of the tests.

The ABR tank was calibrated by measuring the pressure gradient at each location. While experiments were conducted using a measure of acoustic pressure, particle motion calibrations were also calculated from the pressure gradients. Table

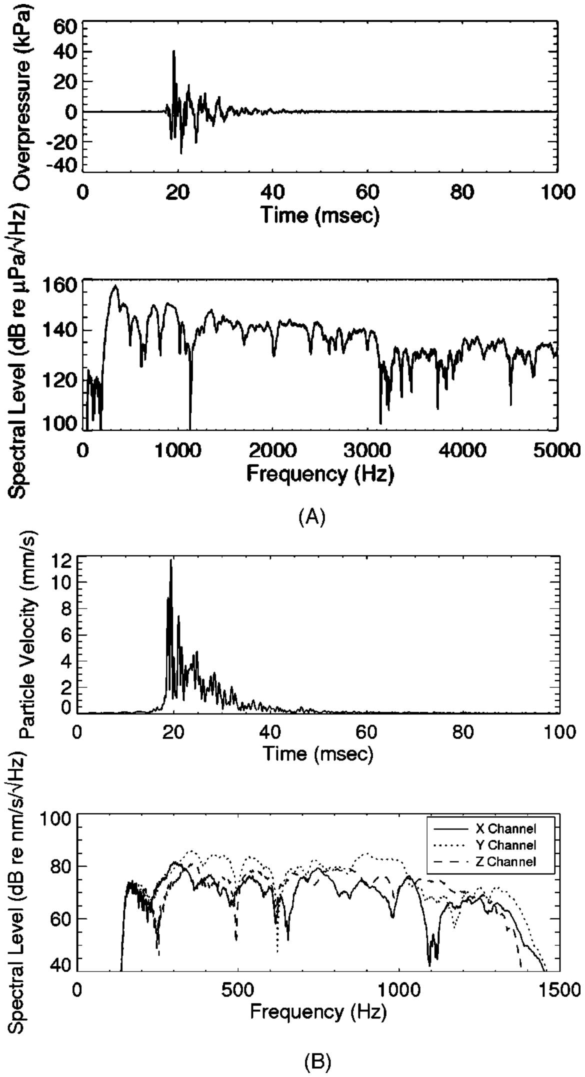

FIG. 2. (A) Acoustic pressure wave form and spectral levels for a single airgun array shot as measured during the noise exposure tests. (B) Particle velocity amplitude (top) and single-channel velocity spectral levels (bottom) as measured for a single airgun array shot during the noise exposure tests.

II shows the relationship between pressure and particle motion for each frequency at the head of the fish. There is a clear indication of a strong particle motion signal at each frequency tested. However, since it is not known whether the three species were detecting particle motion, pressure, or a combination of both signals, it is impossible to present hearing data in terms of which signals are most relevant to each species. At the same time, since there is a strong particle motion component at each frequency tested, any threshold shifts encountered most likely reflect a loss of detection capabilities of fishes to the signal(s) most relevant to their own hearing.

TABLE II. Calibration data for test tank showing particle velocity magnitudes corresponding to a 100 dB re 1 μPa sound pressure.

Frequency	Lake chub particle velocity (dB re 1 nm/s)	Pike/Whitefish (dB re 1 nm/s)
100	87	72
200	61	58
400	72	67
800	53	56
1600	24	12

Statistical analysis

The effect of exposure to seismic airgun noise and recovery from the exposure on auditory threshold levels were tested using separate ANOVAs with treatment (control or noise-exposed) and frequency as factors. Tukey's *post-hoc* test was used to make pairwise comparisons between treatments at specific frequencies when significant main effects were found with the overall ANOVA (Zar, 1998). SYSTAT (version 10) was used for all statistical tests.

RESULTS

We first provide general observations of hearing thresholds for each species examined (baseline data are presented in Mann *et al.*, unpublished) and then describe the effect of exposure to seismic airguns for each species separately. It should be noted that since we were collecting animals in the wild, we had little control over fish size. Analysis of size data relative to hearing thresholds, however, showed no difference in hearing sensitivity between fishes of the same species within the size ranges used (Mann *et al.*, unpublished). Consequently, data for all animals in each experimental and control group were pooled in presenting results. The only data not pooled were those for adult and YOY northern pike. While their hearing thresholds were similar between these two groups, the size differences in the specimens used were so great that it was decided to keep the groups separate in all data analyses.

Control hearing thresholds showed that lake chub has far more sensitive hearing and broader bandwidth of hearing than broad whitefish or northern pike (Figs. 3, 4, 6). This is as expected since the lake chub is a member of the superorder Otophysi, a group of hearing specialists that have a set of bones, the Weberian ossicles, which acoustically couple the swim bladder to the saccule of the inner ear. Hearing sensitivity is greater in the northern pike than the broad whitefish.

Our initial analysis of hearing sensitivity in the northern pike and broad whitefish showed that they could detect sounds up to 1600 Hz (the highest frequency used in this study). However, both species had very poor hearing at 800 and 1600 Hz. Since the speaker used in the ABR studies could not produce amplitudes that would be much higher than normal thresholds at 800 and 1600 Hz, it was not possible to examine hearing loss at these frequencies since we would have had to generate signals well above threshold to evaluate hearing loss (a higher hearing sensitivity). Thus, we chose to only test for threshold shift at 100, 200, and 400 Hz for these two species. Threshold shift was measured to 1600 Hz for the lake chub since its hearing bandwidth is greater and its normal thresholds are well below those of the other species.

General observations

While we did not do a standard necropsy or histopathology on test animals, general examination of the external

TABLE III. Hearing thresholds for broad whitefish controls and experimental animals exposed to five seismic shots.

Frequency	Mean	SD	SE	N	Frequency	Mean	SD	SE	N
Control					Experimental: 5 shots, tested shortly after exposure				
100	114.8	13.99	5.29	7	100	115.7	5.02	2.24	5
200	112.8	9.47	3.58	7	200	108.5	5.02	2.24	5
400	113.1	18.31	6.92	7	400	109.7	11.54	5.16	5

anatomy post-exposure did not show any apparent effect of noise exposure as compared to controls. In addition, we did not note any bleeding or other overt effects on the eyes, gills, or internal organs in experimental or control groups when they were dissected in preparation for preservation of ear tissue for later analysis (Popper *et al.*, unpublished). The swim bladders were fully intact and inflated in all experimental and control specimens of all three species. Moreover, fish swam normally post-exposure and all fish that we maintained for use 24 hours post-exposure survived with no apparent adverse effects.

Broad whitefish

Fish ranged in size from 350 to 510 mm in standard length and 735 to 2810 grams in weight. Hearing was only measured immediately post-exposure due to difficulties in keeping these very sensitive animals alive in captivity. Hearing thresholds were obtained from five experimental fish and seven controls (Table III). Thresholds of whitefish exposed to five airgun shots were not significantly different from those of controls ($F = 0.31$, $P = 0.58$; Fig. 3), indicating that the airguns had no apparent effect on hearing in this species. There were no exposure effects on mortality.

Northern pike

We measured two groups of northern pike. One included adults from 360 to 670 mm in standard length and 430 to 2460 grams in weight. The second group included YOY fish that ranged from 70 to 110 mm in standard length and 1.7 to 8.8 grams in weight.

Adult pike exposed to five airgun shots exhibited mean thresholds that were higher than controls ($F = 36.31$, $P < 0.0001$), although this was significant only at 400 Hz ($P = 0.0003$) because of low power ($N = 4$ or 7; power $= 80\%$, 66%, and 99% for 100, 200, and 400 Hz, respectively; Fig 4(A), Table IV). We plotted the threshold shift (exposed-control thresholds) to visualize trends in hearing loss across frequencies [Fig. 4(B)]. The greatest threshold shift was approximately 20 dB at 400 Hz. However, 18 hours after exposure to the airguns, the thresholds for northern pike were no longer significantly different from controls ($P > 0.60$ for all frequencies), indicating complete recovery from hearing loss [Fig. 4(A)]. In contrast to the threshold shifts exhibited by adult pike, juvenile pike exhibited no hearing loss after being exposed to either five or 20 airgun shots ($P > 0.10$, Fig. 5, Table V).

Lake chub

The response of lake chub (Table VI) was tested for both five and 20 airgun shots (Fig. 6) and for recovery from both signals (Fig. 7). Fish tested shortly after exposure to five airgun shots showed statistically significant threshold shifts at 200 ($P < 0.0001$), 400 ($P = 0.018$), and 1600 Hz ($P = 0.001$). Lake chub that received 20 shots and then tested shortly after exposure showed hearing thresholds that were statistically different from controls ($P \leqslant 0.001$ for all frequencies). There was a mean difference in thresholds between animals exposed to five shots compared to those exposed to 20 shots ($F = 57.08$, $P < 0.0001$), but these differences were only significant at 400 Hz ($P < 0.0001$) and 800 Hz [$P = 0.027$; Fig. 6(A)]. The greatest threshold shifts

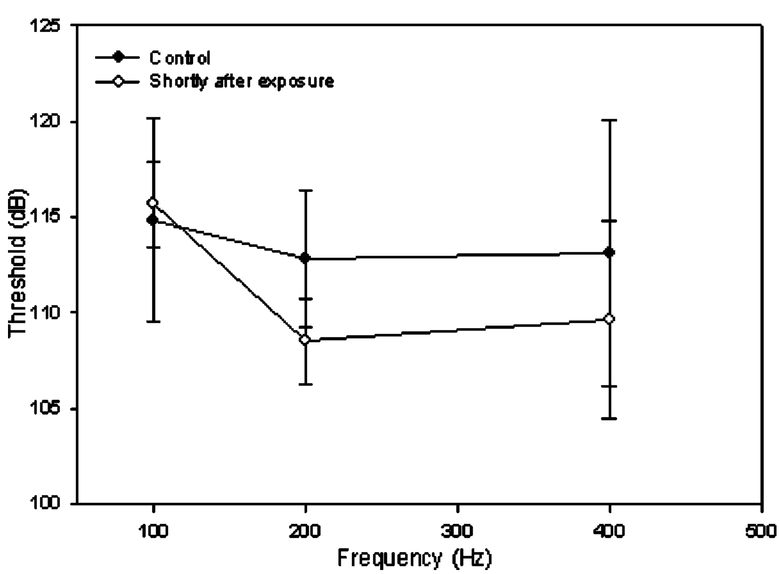

FIG. 3. Broad whitefish experimental vs. control data (mean ± SE) for fish exposed to five airgun shots.

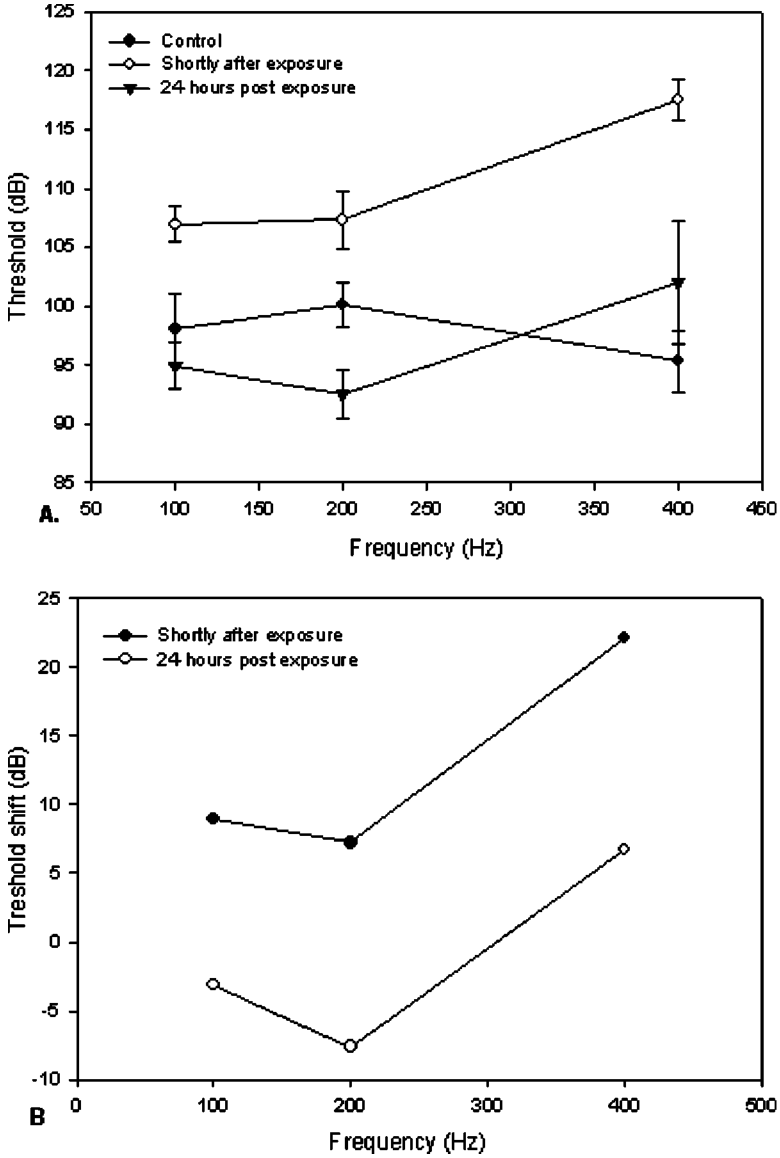

FIG. 4. (A) Thresholds for adult northern pike for controls and those exposed to five airgun shot immediately and 24-hours post-exposure. (B) Data for adult northern pike expressed as experimental threshold minus control data to show the effects of airgun exposure. Positive values indicate a hearing loss.

to five shots and 20 shots were approximately 25 dB at 200 Hz and 35 dB at 400 Hz, respectively [Fig. 6(B)]. Lake chubs tested 18 hours after exposure to five airgun shots had thresholds close to those of controls held for 18 hours (although this was not tested statistically because of low sample size) [Fig. 7(A)]. Chubs exposed to 20 airgun shots showed full recovery from hearing loss and had thresholds that were not significantly different from controls [$F=0.18$, $P=0.67$; Fig. 7(B)].

DISCUSSION

This study represents the first physiological evaluation of the effects of airgun use on the hearing sensitivity of fish. Earlier studies evaluating the effects of continuous noise and pure tones on hearing have shown temporary threshold shift (TTS) (e.g., Popper and Clarke, 1976; Scholik and Yan, 2001, 2002; Smith *et al.*, 2004a, 2004b). Such studies led to the concern that exposure to very intense sounds, such as

TABLE IV. Hearing thresholds for adult northern pike controls and experimental animals exposed to five seismic shots tested immediately post-exposure and another group tested 24 hours post-exposure.

Frequency	Mean	SD	SE	*N*	Frequency	Mean	SD	SE	*N*
Control					Experimental: Five shots, tested shortly post-exposure				
100	98.0	7.78	2.94	7	100	107.0	3.00	1.50	4
200	100.1	4.95	1.87	7	200	107.3	4.90	2.45	4
400	95.3	6.85	2.59	7	400	117.5	3.46	1.73	4
					Experimental: Five shots, tested 24 hours post-exposure				
					100	94.9	3.46	2.00	3
					200	92.5	3.46	2.00	3
					400	102.0	9.17	5.29	3

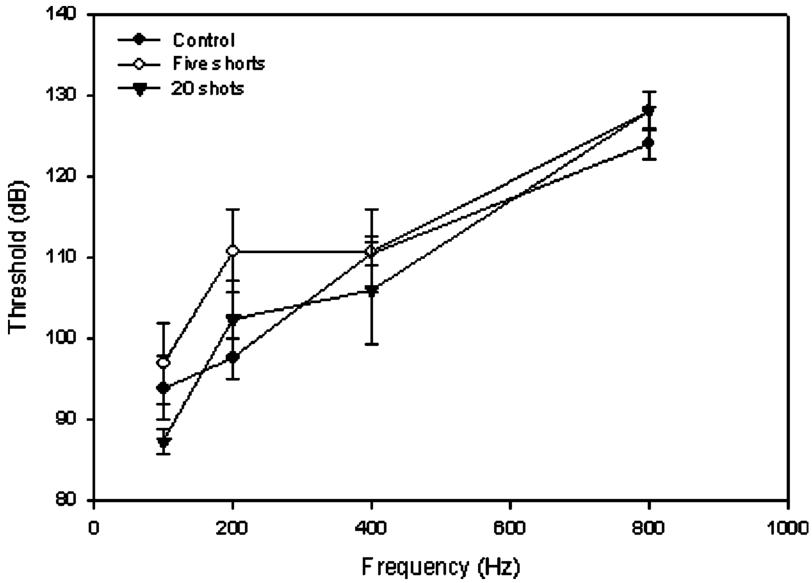

FIG. 5. Response of YOY northern pike to five and 20 airgun shots as compared to controls (mean±SE). There is no statistical difference between exposed and control animals, indicating that airgun exposure did not affect hearing in these animals.

those from airguns, could impair fish hearing. At the same time, the sounds from an airgun are strikingly different from those used in earlier studies in several respects. Perhaps most importantly, unlike the sounds used in earlier studies, the sound from the airguns have an extremely rapid onset, contain energy over a broad frequency range, and have a much higher peak sound level (Fig. 2) than the noise or pure tones used in other studies. Thus, the sounds of airguns are closer to those of pile driving and explosions than sounds of ship noise or sonar.

The results of three fish species from the Mackenzie Delta demonstrate that there are substantial differences in the effects of airguns on the hearing thresholds of different species. Interestingly, the effects appear to have a correlation with hearing sensitivity of the fish. Thus, the broad whitefish, the species with poorest hearing sensitivity as measured in our apparatus, showed no apparent effects from the airgun exposure (five shots), while the lake chub, the species with most sensitive hearing, showed the most effect to both five and 20 airgun shots. The northern pike has hearing sensitivity that is between the two other species (albeit closer to that of the whitefish) and adult pike showed statistically significant hearing loss but less than that encountered with the chub. For reasons that we do not understand, however, YOY northern pike did not show any statistically significant hearing loss as a result of airgun exposure, even to 20 shots, although this may be related to issues associated with development of the auditory system (e.g., Kenyon *et al.*, 1998; Wysocki and Ladich, 2001).

The sound spectrum of the airgun array shots (Fig. 2) in the riverine exposure showed most energy above 300 Hz. While the spectrum of airguns can be expected to contain a large low frequency component (e.g., McCauley *et al.*, 2003), there is considerable loss of low frequency energy in shallow waters (e.g., Rogers and Cox, 1986), thereby resulting in a difference in the spectral components of airguns events in waters of different depths. Significantly, the spectrum of the airguns used in this study generally matched the threshold shifts observed in adult northern pike and in lake chub. Both of these species showed the greatest threshold shifts at 400 Hz, with less hearing loss at 100 Hz and 200 Hz. However, the lake chub did show large threshold shifts at 200 Hz, even though there was relatively little energy at 200 Hz in the shot.

There are suggestions in the literature that the effects of high intensity sound on the hearing abilities of fish are related to the level of the stimulus sound above the threshold of the fish (Hastings *et al.*, 1996; Smith *et al.*, 2004a, 2004b). It has been hypothesized that noise-induced threshold shifts in fish are linearly related to the sound pressure

TABLE V. Hearing thresholds for YOY northern pike controls and experimental animals exposed to five seismic shots and 20 seismic shots.

Frequency	Mean	SD	SE	*N*	Frequency	Mean	SD	SE	*N*
YOY pike: controls					YOY pike: five shot tested shortly post-exposure				
100	93.9	7.75	3.87	4	100	96.9	11.22	5.02	5
200	97.6	4.90	2.45	4	200	110.8	11.54	5.16	5
400	110.5	3.00	1.50	4	400	110.8	11.54	5.16	5
800	124.0	3.46	1.73	4	800	128.2	5.02	2.24	5
					YOY pike: 20 shots, tested shortly after exposure				
					100	87.3	3.29	1.47	5
					200	102.4	10.73	4.80	5
					400	106.0	14.70	6.57	5
					800	128.2	5.02	2.24	5

TABLE VI. Hearing threshold for lake chub controls tested immediately and controls tested after 18 hours and for experimental animals exposed to five and 20 shots tested immediately and 18 hours post-exposure.

Frequency	Mean	SD	SE	*N*	Frequency	Mean	SD	SE	*N*
Control					Experimental: 5 shots, test shortly post-exposure				
100	80.4	5.20	2.33	5	100	94.1	5.02	2.24	5
200	73	3.82	1.71	5	200	97.7	7.82	3.50	5
400	70.9	10.29	4.60	5	400	87.2	7.82	3.50	5
800	70.4	6.68	2.99	5	800	90.9	7.82	3.50	5
1600	89.7	7.63	3.41	5	1600	101.1	4.24	1.90	5
Control: fish kept 18–24 h post-exposure					Experimental: 5 shots, tested 18 h post-exposure				
100	87.6	7.77	3.17	6	100	89.9	4.24	3.00	2
200	79.2	10.50	4.29	6	200	87.5	4.24	3.00	2
400	77.3	4.33	1.77	6	400	83.0	4.24	3.00	2
800	82.5	6.93	2.83	6	800	83.7	0.00	0.00	2
1600	92.6	6.99	2.85	6	1600	89.1	8.49	6.00	2
					Experimental: 20 shots, tested shortly post-exposure				
					100	100.4	3.00	1.50	4
					200	110.0	3.00	1.50	4
					400	111.5	5.74	2.87	4
					800	104.7	3.46	1.73	4
					1600	108.6	3.00	1.50	4
					Experimental: 20 shots, tested 18 h post-exposure				
					100	93.5	7.63	2.89	7
					200	77.0	7.63	2.89	7
					400	77.7	7.52	2.84	7
					800	80.7	11.86	4.48	7
					1600	94.8	8.38	3.17	7

difference (SPD) between the sound pressure of the noise and the baseline hearing threshold of the fish (called the linear threshold shift, or LINTS hypothesis; Smith *et al.*, 2004b), as has been found in birds and mammals. Since the baseline thresholds of fish vary with frequency, this difference is calculated separately for each frequency tested. The linear TTS relationships of Smith *et al.* (2004b) were found after exposing fish to continuous sounds. In order to examine if this LINTS relationship is valid for more impulsive, short duration sounds such as our seismic airgun stimulus, we plotted our TTS data against SPD. The sound level used to calculate SPD was the measured 90% RMS SPL (dB re 1 μPa) of the airgun shots (see Table I). Regression analysis was used to examine the relationship between SPD and TTS.

Lake chub exposed to five and 20 airgun shots both exhibited a significant linear relationship between SPD above baseline hearing thresholds and TTS ($P<0.0001$; Fig. 8). Lake chub that were exposed to 20 airgun shots had TTSs that were greater than those exposed to only five airgun shots (see Results), and their LINTS relationship had a slightly greater slope as shown by a significant SPD and shot treatment interaction ($F=3.53$, $P=0.016$; Fig. 8).

The LINTS relationship is more evident for lake chub, which are hearing specialists with lower baseline hearing thresholds, than for pike and whitefish, which are hearing generalists with higher baseline hearing thresholds. As a result of the differences in hearing capabilities between these two groups, the SPD of our airgun source above baseline hearing thresholds is generally greater for chubs and minimal in pike and whitefish. Despite this fact and the low number of frequencies tested, adult pike exhibited a significant LINTS relationship (TTS=0.53, SPD=45.50; $R^2=0.21$, $P=0.040$), although whitefish did not ($P=0.067$). When all three species are plotted simultaneously, a significant LINTS relationship exists (Fig. 9).

This finding supports the LINTS hypothesis and suggests that such a relationship is valid for TTS induced by both continuous and impulsive sound sources. The result is a predictable linear relationship between SPD above baseline thresholds and TTS for these three species with varying hearing capabilities (Fig. 9). These findings support the idea that the LINTS relationship may ultimately be usable by fisheries managers attempting to mitigate the effects of intense anthropogenic sounds on fishes. In general, the only information that fisheries managers would need to predict such relationships is the audiogram of the species of interest and the sound spectrum, level, and duration of the sound stimulus in question.

While we did not attempt to use different levels of airgun sounds, we were able to use different total energy exposure in both the lake chub and northern pike, and this is equivalent to the effects of higher sound levels for noise studies if one assumes that hearing loss is a response to total energy impinging upon the animal, as predicted by the LINTS hypothesis. Our findings show that as the total energy of exposure increases (20 versus five airgun shots), there is a substantial increase in TTS in lake chub but not in northern pike YOY (which showed no TTS to any sounds).

Clearly, it would have been useful to have tested the LINTS idea with broad whitefish, as the LINTS hypothesis and the results with lake chub and northern pike suggest that

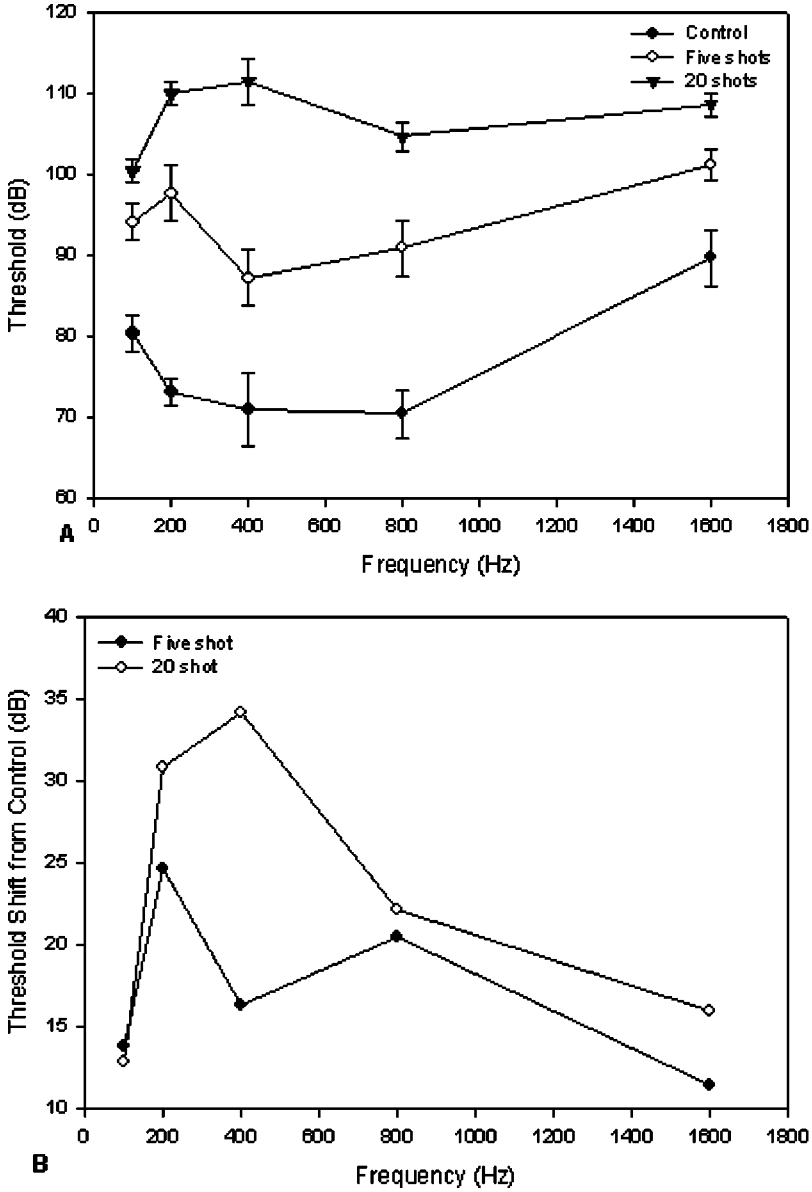

FIG. 6. Noise exposure data for lake chub exposed to five and 20 airgun shots. (A) Threshold data for controls and animals tested immediately after exposure. (B) Data expressed in terms of difference between exposure and threshold hearing levels.

had whitefish been exposed to 20 airgun shots they might have demonstrated TTS in that species as well. However, this could not be done since insufficient specimens were available.

Despite the presence of a hearing loss immediately post-exposure, tests on lake chub and northern pike, 18 to 24 hours post-exposure, respectively, to five airgun shots shows a return to about normal thresholds. Thus, the hearing loss encountered after exposure appears to be temporary threshold shift, although any final assessment of whether there is longer term damage that shows up later post-exposure will have to await microscopic analysis of the ear tissue (Popper *et al.*, unpublished).

There were no obvious trends in the hearing measurements made from a group of exposed animals over the 1.5 hours it took to test them. It would be of considerable interest for future studies to examine the time course of recovery, but this would require the experimenters to be able to expose and test fish on a very tight time schedule—something not possible during the course of these experiments.

One concern is that our results show that exposure to 20 airgun shots is very likely to cause a TTS in every species exposed, except juvenile northern pike. It is unlikely that fish would encounter 20 shots in a river seismic survey unless they were ‘‘herded’’ with the survey vessel. Only strong swimming fish such as adult broad whitefish would be capable of being ‘‘herded’’ by the sound source and potentially stay in the vicinity of the airguns over multiple emissions. Thus the test with 20 shots over 15 minutes at 210 dB re 1 μPa probably represents an extreme exposure for fishes in the Mackenzie River Delta. The actual exposure of fish to a seismic survey depends on the speed of the survey vessel and the movements of the fish (Jorgensen *et al.*, unpublished). To understand the actual exposure will require behavioral studies on the movements of fishes in response to airgun surveys.

The results of this study have implications for airgun surveys, particularly in riverine situations. Our experimental paradigm was designed around a 2D survey that might be conducted in a river where the airgun vessel steadily moves in one direction, as opposed to off-shore 3D surveys where

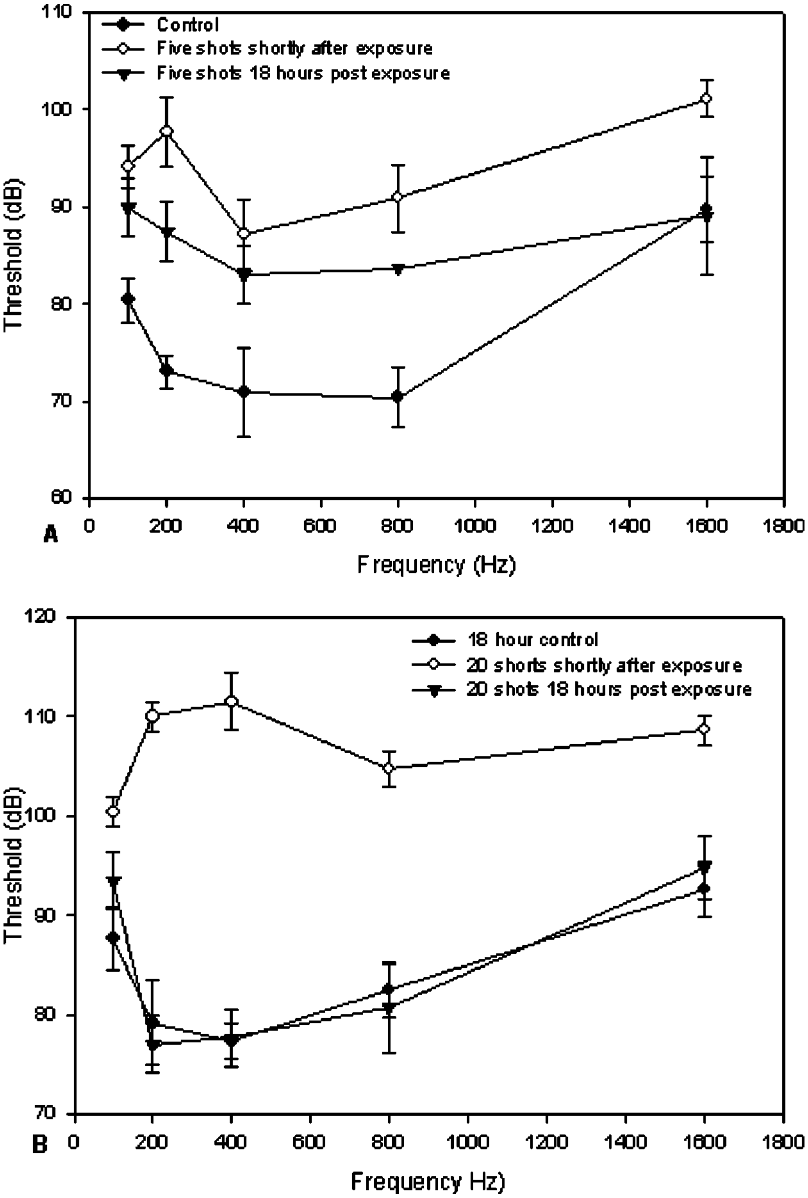

FIG. 7. Recovery of lake chub from exposure to five (A) and 20 (B) airgun shots.

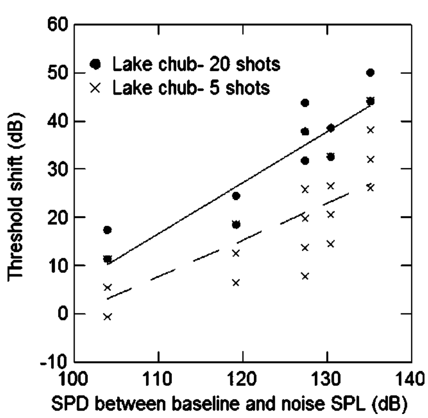

FIG. 8. Relationship between sound pressure difference (SPD) between the noise level and baseline hearing thresholds and temporary threshold shifts (TTS) for lake chub exposed to 5 or 20 shots of the airgun. The dashed and solid lines represent the linear regression relationships for chub exposed to 5 ($TTS = 0.76\ SPD - 75.84$, $R^2 = 0.57$, $P < 0.001$) and 20 airgun shots ($TTS = 1.06\ SPD - 99.44$, $R^2 = 0.83$, $P < 0.001$), respectively. Each data point represents the TTS ($N = 4-5$) at each of the five frequencies tested.

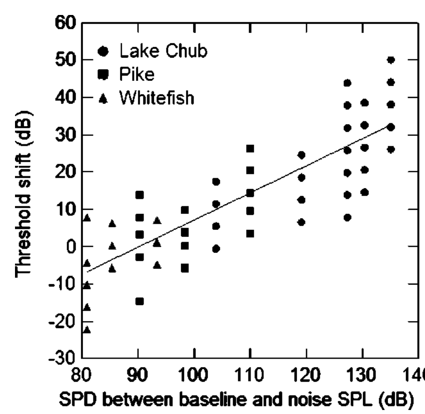

FIG. 9. Relationship between sound pressure difference (SPD) between the noise level and baseline hearing thresholds and temporary threshold shifts (TTS) of adult northern pike, lake chub, and broad whitefish exposed to the airgun. The solid line represents the linear regression relationship for all three species ($TTS = 0.53\ SPD - 48.40$, $R^2 = 0.40$, $P < 0.0001$). Each data point represents the mean TTS ($N = 4-5$) at each of the frequencies tested.

the airguns are often towed back and fourth over parallel tracks (Bott, 1999). Fish in a river are exposed to airguns as the tow boat approaches and then passes by. In contrast, in a 3D off-shore seismic exploration program, a resident fish may be exposed to the airgun over and over again as the seismic array is moved across parallel paths.

In design of our study, we presented fish with five exposures to the airgun at 40 second intervals so that the fish were exposed to a steady sound level (Table I). In contrast, a normal survey might present signals as often as every 10 seconds (this could not be done in this study due to limitations of the compressor used to charge the guns). Since a seismic survey vessel is moving, a stationary fish subject is exposed to the maximum level once in a sequence of exposures. Moreover, the majority of exposed fishes during a seismic survey are likely to be at greater distances from the source than those in this study and thus receive a somewhat lower sound level. Though these factors do not compensate for the more frequent exposure in an actual survey, without use of a seismic vessel for experiments of this sort it is likely that our experiments presented fish with an approximate ''worst case'' with regard to seismic stimulation.

Based upon this being a ''worst case'' and the differences in effects on different species, it may be possible to suggest some general concepts with regard to seismic stimulation. First, it appears that it may be possible to predict whether a fish will show hearing loss as a consequence of exposure to airgun noise based upon baseline hearing thresholds of a particular species and using the LINTS determinations. Fish with poorer hearing in this study, such as pike, showed little hearing loss, while the fish with the best hearing, the lake chub, had the most loss.

Second, our data indicate recovery of hearing loss within 18 and 24 hours in the lake chub and northern pike, respectively, even after exposure to 20 airgun shots. It is unclear why complete recovery did not occur in the two chubs exposed to five airgun shots, and more data are needed to draw conclusions about recovery. However, despite recovery it is important to note that during the period of TTS fish may still be impaired in their ability to survive since they would have some loss of their ability to hear biologically relevant sounds.

Comparison with other airgun studies

The only other experimental airgun study that looked directly at the effects on fish physiology was done by McCauley *et al.* (2003) using a different paradigm and different species than used here. Thus, a direct comparison between the two studies is tenuous at best. The McCauley *et al.* study only looked at effects on ear tissue and did not examine the hearing ability of the experimental species, the pink snapper, and there are no data on hearing capabilities of that species. At the same time, McCauley *et al.* reported a profound and long-lasting effect on the sensory cells in the saccule of the ear, and it remains to be seen whether the same effect is found in this study (Popper *et al.*, unpublished).

There are several other differences in the two studies that make it hard to compare results. First, McCauley *et al.* (2003) used a 330 cc (20 in.^{3}) single airgun as compared to the 12 000 cc (720 in.^{3}) array used in this study. McCauley *et al.* also exposed fish with frequent emissions in two periods of almost 1 hour each to partially mimic the kinds of exposure fish might get were they stationary and exposed to an airgun that moves back and forth, as happens in 3D marine surveys. Thus, the cumulative exposure to the airgun in the McCauley *et al.* study may have been greater than in this study. Second, the McCauley *et al.* study was in deeper water and so the spectrum of the sounds to which the fish were exposed in the two studies differed somewhat, with there being more energy below 300 Hz in McCauley *et al.* than in the current investigation.

It clearly would be important to replicate the McCauley *et al.* (2003) study to ascertain effects on hearing, as well as on different fish species. Similarly, it would be of value to replicate our study using even greater stimulus levels or higher exposure rates in order to test whether the fish exposed to such sounds would ultimately show permanent hearing loss that would be associated with the loss of sensory cells found by McCauley *et al.* (2003).

Caveats and future studies

The results from this study provide a qualitative model to predict when exposure to airguns may have an effect on hearing capabilities of fishes. However, there are several caveats that must accompany our results before they are broadly applicable to other studies. We also suggest that additional studies need to be conducted to help resolve the remaining issues.

First, while we studied fishes with different hearing capabilities, since there is substantial diversity in the structure of the auditory systems of different species (e.g., Popper and Carlson, 1998; Popper *et al.*, 2003), it would be of use to look at the effects of airguns on species with other hearing specializations.

Second, while our results support the LINTS hypothesis in suggesting that increased total noise exposure will result in increased hearing loss, this idea needs to be tested more directly with airgun exposures of different total energy levels, including different numbers of airgun shots.

Third, it is not clear whether an increase in total noise exposure will result in permanent hearing loss (permanent threshold shift, PTS), and how the results reported here fit with the recent work of McCauley *et al.* (2003) that showed significant inner ear damage as a result of noise exposure that probably exceeded any used here. Future studies from our laboratory will evaluate ear structure in the Mackenzie River animals, but due to the nature of the species selected and our holding facilities at Inuvik, it was not possible to hold fish for the extended periods of time used by McCauley *et al.* It would be of considerable value to replicate our studies but then hold fish for many days and weeks post airgun exposure to determine if there is damage to the auditory system and if this is manifest in late onset hearing loss.

Fourth, our work was done in a river using an exposure paradigm designed to mimic a single pass exposure to a seismic source. In order to compare to the impacts of a 3D seismic survey, it will be important to replicate our type of behavioral study using a sound exposure paradigm more like that used by McCauley *et al.* (2003) to mimic repeated exposure to sound.

Fifth, it must be kept in mind that this study was done in a river, with the fish in 1.9 m of water. Sound propagates differently in shallow water than it does in deep water, with much less propagation of low frequency energy in shallow water (Rogers and Cox, 1986). It is therefore important to determine if the effects of airgun sound on fish are the same in deep water as they are in shallow water.

Finally, we caution that the results reported here, while highly informative, are not the final word on the effects of airguns on fishes. And they are clearly *not* applicable to the potential effects from other anthropogenic sources such as shipping, sonar, or other more or less ''continuous'' signals or signals that do not have rapid onsets. Moreover, extrapolation to other species must still be done with considerable caution.

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