The inner ears of Northern Canadian freshwater fishes following exposure to seismic air gun sounds

Jiakun Song\textsuperscript{a)}
Department of Biology, Neuroscience and Cognitive Science Program, and Center for Comparative and Evolutionary Biology of Hearing, University of Maryland, College Park, Maryland 20742 and Institute for Marine Biosystem and Neurosciences, Shanghai Fisheries University, Shanghai 200090, China

David A. Mann\textsuperscript{b)}
College of Marine Science, University of South Florida, 140 7th Avenue South, St. Petersburg, Florida 33701

Peter A. Cott\textsuperscript{c)} and Bruce W. Hanna\textsuperscript{d)}
Fisheries and Oceans Canada, 101, 5204-50th Avenue, Yellowknife, Northwest Territories X1A 1E2, Canada

Arthur N. Popper\textsuperscript{e)}
Department of Biology, Neuroscience and Cognitive Science Program, and Center for Comparative and Evolutionary Biology of Hearing, University of Maryland, College Park, Maryland 20742

(Received 5 September 2007; revised 5 April 2008; accepted 28 May 2008)

An earlier study examined the effects of exposure to seismic air guns on the hearing of three species of fish from the Mackenzie River Delta in Northern Canada [Popper et al. (2005). “Effects of exposure to seismic airgun use on hearing of three fish species.” J. Acoust. Soc. Am. 117, 3958–3971]. The sound pressure levels to which the fishes were exposed were a mean received level of 205–209 dB re 1 \( \mu \)Pa (peak) per shot and an approximate received mean SEL of 176–180 dB re 1 \( \mu \)Pa\(^2\) s per shot. In this report, the same animals were examined to determine whether there were effects on the sensory cells of the inner ear as a result of the seismic exposure. No damage was found to the ears of the fishes exposed to seismic sounds despite the fact that two of the species, adult northern pike and lake chub, had shown a temporary threshold shift in hearing studies.

© 2008 Acoustical Society of America. [DOI: 10.1121/1.2946702]

PACS number(s): 43.80.Lb, 43.80.Nd, 43.64.Wn [MCH] Pages: 1360–1366

I. INTRODUCTION

There is growing concern that human-generated (anthropogenic) sounds may have an impact on the health and/or survival of fishes (e.g., Myrberg, 1980; Popper, 2003; Popper et al., 2004; Hastings and Popper, 2005). The types of sounds of interest vary greatly in their acoustic parameters but include noise from shipping, low- and midfrequency sonars, pile driving, and a range of other anthropogenic sources.

One of the anthropogenic sources of primary concern has been air guns used in seismic surveys. These are used extensively by the oil and gas industry for exploration and by geologists for subsea studies. Although most work with air guns has taken place in marine environments, there has been an increase in seismic exploration in rivers and lakes, and as a result, concerns have expanded to include freshwater fish species.

Air guns produce a compressed air bubble that, once released, collapses under the pressure of water. This results in a sharp concussive sound with peak sound levels possibly equal to or exceeding 230 dB (re 1 \( \mu \)Pa) at 1 m from a single air gun. The general procedure for this type of seismic survey is to trail an air gun array behind a boat and set off frequent “shots.” These sounds are directed down toward the substrate and reflect off geologic formations below the water-substrate interface. The reflected signals are picked up by hydrophones that are towed behind the vessel or laid on the bed of the waterbody (Bott, 1999).

Although it has been suggested that the sound energy from air guns may harm fishes (reviewed by Popper, 2003; Popper et al., 2004), there are few studies that have directly examined the potential effects on fish physiology or behavior. McCauley et al. (2003) found that exposure of caged pink snapper (Pagrus auratus) to multiple emissions of a single seismic air gun resulted in substantial damage to the sensory cells in a small region of the saccule, one of the end organs in the inner ear of fishes. McCauley et al. (2003) also found that although some damage was found several hours after exposure, the damage to the sensory tissue apparently continued to increase in the days postexposure, although it never was found over more than a small portion of any saccular epithelium.
More recently, Popper et al. (2005) examined the effects of exposure to a 730-in$^3$ seismic air gun array on the hearing capabilities of several different fish species found in the Mackenzie River near Inuvik, Northwest Territories, Canada (see map by Popper et al., 2005; Mann et al., 2007). The investigators showed that there was some temporary hearing loss as a result of air gun exposure in lake chub (Couesius plumbeus) and adult (but not young of the year) northern pike (Esox lucius) but no effects on hearing in broad whitefish (Coregonus nasus). All three fishes are freshwater species and represent the first nonmarine species tested for effects from seismic air gun noise. In addition, the broad whitefish is an important subsistence fish in the region, and there is concern that damage to this species could have impacts to subsistence harvesting.

This paper extends the work of Popper et al. (2005) by examining the inner ear tissues of the same specimens that were exposed to air gun sounds in that study. In addition, since the ears of these three species have not yet been described, their anatomy and ultrastructure are presented briefly.

II. METHODS

Animals were obtained from the Mackenzie River and held at the Fisheries and Oceans Canada facilities in Inuvik, Northwest Territories. Fish capture, holding methodology, and seismic exposure were described elsewhere (Popper et al., 2005). Fish were exposed to 5 or 20 shots from a 730-in$^3$ air gun array to emulate an exposure comparable to what fish would experience from a seismic survey in a river (see Table I). The mean received sound pressure levels of sounds to which the fishes were exposed for each shot were from 205 to 209 dB re 1 $\mu$Pa (peak), which was equivalent to an approximate mean received level per shot sound exposure level (SEL) of 176–180 dB re 1 $\mu$Pa$^2$ s. Particle motion levels of the received sound are presented by Popper et al. (2005).

Fishes were exposed to air guns and then examined for hearing capabilities prior to the ears being fixed for ultrastructural examination (Popper et al., 2005). Ears were examined from each exposed species (broad whitefish, lake chub, and adult and young northern pike). The experimental groups and number of animals in each group are presented in Table I.

Within 15 min following hearing tests, the fish were euthanized with an overdose of buffered tricaine methane sulfonate (MS-222). The heads were quickly opened and fixative (4% gluteraldehyde and 2.5% paraformaldehyde in buffer, pH 7.4) was squirted into the cranial cavity. The brain was then carefully removed and the extraneous tissue was dissected away, and at all times, the ear region was kept wet with fixative. The ear regions were then placed in jars of cold fixative and shipped overnight to the University of Maryland where the ears were dissected and examined.

Once at the University of Maryland, the ears were then removed from the heads and photographed in a dissection microscope, and the otoliths were then removed. The tissue was then washed in three changes of phosphate-buffered saline (PBS) (0.1M, pH 7.4), postfixed in 1% osmium tetroxide (in PBS), and further dissected to reveal the sensory epithelia of the three otolithic end organs, the saccule, lagena, and utricle (Fig. 1). The tissue was then dehydrated through a graded ethanol series (30%, 50%, 70%, 80%, 90%, and 95%)
and up to three changes of 100% ethanol. The tissue was subsequently critical point dried using liquid CO2 as the intermediary fluid, mounted on aluminum stubs, coated with a 25-nm-thick layer of gold/palladium. 

These types of damage have been seen in earlier studies (e.g., Enger, 1981; Hastings et al., 1996; McCauley et al., 2003).

III. RESULTS

A. General ear structure

The ears of fishes include three semicircular canals and three oolith organs, the saccule, lagena, and utricle (Fig. 1). Each of the oolithic organs has a single dense calcareous otolith that lies adjacent to the sensory epithelium. The epithelium contains a large number of sensory hair cells (Figs. 2–6) from which arise ciliary bundles that are made up of a single kinocilium and multiple stereocilia. The ciliary bundles project into the lumen of the otolithic chamber so that the tips of the cilia come close to or contact the otolith. It should be noted that there are cracks in various tissues as in (F). However, these cracks are widely seen in SEM tissue of fish ears (e.g., Popper, 1977) and are artifacts of the fixation and drying process and do not represent effects of noise exposure.
medial and slightly posterior to the saccule [Figs. 1(c) and 1(d)]. The ear of the broad whitefish is very similar to several other salmonids described earlier (Popper, 1976, 1977), and the shape and topographic relationship of saccule and lagena [Fig. 5(a)] are similar to those in the northern pike.

The ears of all species studied lie in the cranial cavity just lateral to the posterior part of the brain [Figs. 1(e) and 1(f)]. Each of the sensory epithelia (including those of the semicircular canals) is innervated by branches of the eighth cranial nerve that then projects into the medulla of the brain.

B. Effects of air gun exposure on experimental fishes

Analysis of each fish showed no damage to any of the sensory tissues of the three otolithic organs (Figs. 2–6). Because there was no apparent damage to any of the fishes, the micrographs presented here were chosen to demonstrate the lack of damage in each species exposed to emissions from air guns, the different numbers of shots to which fish were exposed, and the different time intervals postexposure that fishes were allowed to survive. All sensory epithelia were intact and no differences were observed between baseline controls, controls, and the exposed groups. (For examples of what damage might look like, readers are referred to Enger 1981; Hastings et al., 1996; and McCauley et al., 2003.)

Data for the saccule of northern pike are shown in Fig. 2 for a fish exposed to 5 shots and then sacrificed 1 or 24 h after exposure and for the utricle of young fish exposed to 20 shots [Figs. 6(c) and 6(d)] (Table I). There were no differences in the structure of the sensory epithelia in the experimental fish compared to control and baseline control fish.

Similarly, there was no apparent damage to sensory cells in the lake chub as compared to baseline and control animals [saccule shown in Fig. 3, lagena in Fig. 4, and utricle in Figs. 6(a) and 6(b)]. Moreover, there were no apparent effects in lake chub exposed to 20 shots and allowed to survive for 18 h (e.g., Figs. 2–4 right column) or to fish that received 5 shots and were allowed to survive for 1 h postexposure (Figs. 2–4 left column). Similarly, there was no effect on the utricle of lake chub exposed to 20 shots [Figs. 6(a) and 6(b)].

Finally, there was no damage seen to the saccule and lagena of the broad whitefish exposed to five shots (Fig. 5). No data are available for the utricle.

IV. DISCUSSION

This study examined the gross structure of the ear of three species of freshwater fishes from northern Canada that had been exposed to seismic air guns. The ears of the three species show no distinct differences from the ears of other species studied to date (e.g., Popper et al., 2003; Ladich and
except for a small sac, of unknown function, associated with the ear of the northern pike [Figs. 1(a) and 2(b)]. There is no evidence of any damage to the surfaces and ciliary bundles of the sensory cells of the otolithic organs of any of the species.

A. General ear anatomy

The lake chub has an ear typical of other members of its group, the Otophysi (Popper and Platt, 1983), and is a hearing specialist based on both ear structure and hearing capabilities (Popper et al., 2005). The ear structures support the suggestion that both the northern pike and broad whitefish are hearing “generalists” since neither species has characteristics in gross or ultrastructure that might be found in any hearing specialist (e.g., Popper et al., 2003; Ladich and Popper, 2004). While the function of the small sac associated with the ear of the northern pike is not known, no connection to the swim bladder was observed, and so it is more likely that the chamber is filled with endolymph from the ear than with air. Moreover, hearing data for this species (Mann et al., 2007) supported an argument that the northern pike does not have the hearing range or sensitivity one would expect in a species where there is an air bubble associated with the ear.

B. Effects of seismic exposure

The results show that there was no damage to the sensory epithelia studied in any of the otolithic end organs of any of the three fish species exposed to seismic air guns, including lake chub and adult northern pike held up to 18–24 h postexposure (Table I). In particular, there was no damage to the saccular sensory epithelia, the otolithic end organ of fish thought to be most involved in hearing (Popper et al., 2003). The examined tissues from all exposed fish showed that there were no differences from tissues of controls that were placed in the exposure apparatus but not exposed to air gun noise or from fish that were just kept in holding cages and not moved to the test apparatus. At the same time, both adult northern pike and lake chub exhibited temporary hearing loss (temporary threshold shift; see Popper et al., 2005), showing that hearing loss in fishes is not necessarily accompanied by morphological effects on the sensory hair cells, at least at the level of scanning electron microscopy and at least for the time duration postexposure used in this study.

In contrast to the findings here, several earlier studies have reported damage to sensory epithelia in fishes exposed to pure tones (Enger, 1981; Hastings et al., 1996) and a seismic source (McCauley et al., 2003). In each case, the tissue showed ciliary bundles sheared away from the surfaces of
the sensory cells and/or holes in the epithelia where sensory cells apparently died or were “blown out of” the tissue. None of these effects were seen in the tissues reported here. These results are in notable contrast to the findings of McCauley et al. (2003) that showed substantial morphological damage to the ears of pink snapper. However, as pointed out earlier (Popper et al., 2005), there are significant differences between these two seismic studies including air gun size, the number of air guns, operating pressure to the guns, the sound exposure and recovery time of fishes, and the environment in which the study was conducted. Another difference was the shallower freshwater environment of the Mackenzie River (average of about 1.9 m; Popper et al., 2005) compared with the deeper marine environment of the harbor (Jervoise Bay) in Perth, Australia (average 9 m; McCauley et al., 2003). Due to shallow-water propagation characteristics at the location of the fish cage, there was relatively less low-frequency acoustic energy but more high-frequency acoustic energy in the present study than in the Perth investigation (see power spectra by Popper et al., 2005 and McCauley et al., 2003). It is possible that lower frequency sounds are more likely to elicit damage than higher frequencies, although there are no data to support such an argument. Also, McCauley et al. (2003) found maximum loss of sensory tissue at 54 days postexposure. In contrast, we were only able to examine several species held no more than 24 h postexposure. However, McCauley et al. (2003) also looked at the tissue 18 h postexposure, and although there was no damage at a level that was statistically different from that in control tissue (probably due to small sample size), it was clear that the 18-h tissues were qualitatively very different from those in control tissues. This was not the case in our study where tissues from fish sacrificed postexposure were no different from control and tissues.

ACKNOWLEDGMENTS

This study was supported by Fisheries and Oceans Canada and Indian and Northern Affairs Canada, the Program of Energy Research and Development (PERD), the Inuvialuit Fisheries Joint Management Committee (FJMC), and WesternGeco. Additional support was provided by Grant No. P30 DC-04664 from the National Institute of Deafness and Other Communication Disorders, National Institutes of Health. Numerous people helped with various aspects of this project: Dave Tyson, Marty Bergmann, Don Cobb, Ron Allen, and the DFO Inuvik office staff; Steve Whidden from WesternGeco; Les Harris from the Gwich’in Renewable Resource Board; Kevin Bill, Andrea Hoyt, and the FJMC mentoring program students Gerald Kisoun, Noel Cockney, Candice Cockney, and Angus Alunik; Edward Dillon; Merik Allen; and Ms. Moe Grant who permitted us to use her prop-

FIG. 5. Scanning electron micrographs of the lagena of broad whitefish. Left column [(A), (C), (E), and (F)] Broad whitefish 23C-control. (A) Low-power image of the posterior region of the left saccular epithelium and the left lagena (dashed outlines). (C) High magnification of area in the lagena shown in (A). [(E) and (F)] Higher magnifications of (C), with (E) being more peripheral and (F) more central. Right column [(B), (D), and (G)] Broad whitefish 35E-exposed to 5 shots and sacrificed within 1 h. (B) Right lagena. (D) Left lagena. (G) High magnification of (D). There was no damage to any of this tissue.
FIG. 6. Scanning electron micrographs of utricles of exposed fish. (A) Lake chub 80E exposed to 20 shots at low magnification. (B) Same tissue at higher magnification. (C) Young pike 94E exposed to 20 shots at low magnification. (D) Same tissue at higher magnification. There was no damage to any of this tissue as compared to controls and baseline animals. The poor quality of fixation was found in the utricles of all northern pike used in these experiments.

We thank Dr. Elena Sanovich for considerable help with the images and the plates and Helen Popper for editorial review of the manuscript. Finally, we thank Dr. Mardi Hastings and several excellent reviewers for valuable comments on earlier versions of the manuscript. This study was approved by the Fisheries and Oceans Canada Animal Care Committee and conducted in accordance with Animal Use Permit No. 04-05-009, Scientific Research License No. 13608, and License to Collect Aquatic Plants, Animals and Fish for Scientific Purposes No. SLE-04/05-213.


