High intensity anthropogenic sound damages fish ears

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Marine petroleum exploration involves the repetitive use of high-energy noise sources, air-guns, that produce a short, sharp, low-frequency sound. Despite reports of behavioral responses of fishes and marine mammals to such noise, it is not known whether exposure to air-guns has the potential to damage the ears of aquatic vertebrates. It is shown here that the ears of fish exposed to an operating air-gun sustained extensive damage to their sensory epithelia that was apparent as ablated hair cells. The damage was regionally severe, with no evidence of repair or replacement of damaged sensory cells up to 58 days after air-gun exposure. © *2003 Acoustical Society of America.*
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I. INTRODUCTION

There is a growing concern that intense human-generated (=anthropogenic) sounds in the marine environment may potentially have a substantial impact on marine organisms (e.g., NRC, 1994, 2000; Richardson *et al.*, 1995). Depending upon the magnitude of the signal, there may be no impact on animals or the impact may involve disruption of behavior or even physical or physiological damage to the animals (e.g., McCauley, 1994; Richardson *et al.*, 1995; NRC, 2000).

Much of the interest in the effects of anthropogenic sounds arises from concern for the well-being of marine mammals (e.g., NRC, 2000). However, the marine ecosystem includes a wide range of organisms that detect and use sound for their survival, and their survival is of equal importance to that of marine mammals.

Research on the impact of anthropogenic sounds on non-mammalian species, and particularly fishes, has been extremely limited. The two most relevant studies showed that very high intensity pure tones (e.g., over 180 dB *re* 1 μPa) presented for several hours may cause damage to the sensory hair cells of the ears of several fish species (Enger, 1981; Hastings *et al.*, 1996), while other studies suggested that some sounds will alter the behavior of marine fishes (Engås *et al.*, 1996). Though these investigations hint at potential impacts on fish by anthropogenic sounds, the Engås *et al.* (1996) study is one of the very few that has dealt with anthropogenic sounds that are encountered by wild fishes.

One of the major sources of anthropogenic sounds in the marine environment involves the repetitive use of high-energy noise sources in the water column for marine seismic petroleum exploration. In such investigations, impulsive signals are directed downward and then reflected upwards again by density discontinuities within sub-sea rock strata. The travel times of reflected signals allow geological profiles to be determined. A typical seismic survey may involve many hundred thousand signals spread over several weeks. The most commonly used noise sources are arrays of air-guns that vent high-pressure gas to produce a short, sharp, low-frequency sound (Parks and Hatton, 1986). Although there are references reporting behavioral responses of fishes and marine mammals to seismic survey noise (Pearson *et al.*, 1992; Richardson *et al.*, 1995; Engås *et al.*, 1996), no investigation has been carried out on the potential for damage to the ears of aquatic vertebrates from air-gun exposure.

Here we show that the ears of fish exposed to an operating air-gun that was moved toward and away from the animals sustained extensive damage to their sensory epithelia that was apparent as ablated hair cells. The damage was regionally severe and there was no evidence of repair or replacement of damaged sensory cells up to 58 days after exposure.

II. METHODS

To investigate possible effects of air-gun noise on the hearing system of fishes, we carried out trials where pink snapper (*Pagrus auratus*) held in cages were exposed to signals from an air-gun towed toward and away from the cages, mimicking the stimulus from a passing seismic vessel. Control fish (group I) were kept in the same cages used for experimental animals but were removed from cages and sacrificed just before air-gun stimulation. Group II fish were sacrificed 18 h after air-gun stimulation, and group III fish were sacrificed 58 days later. Air-gun stimulation involved

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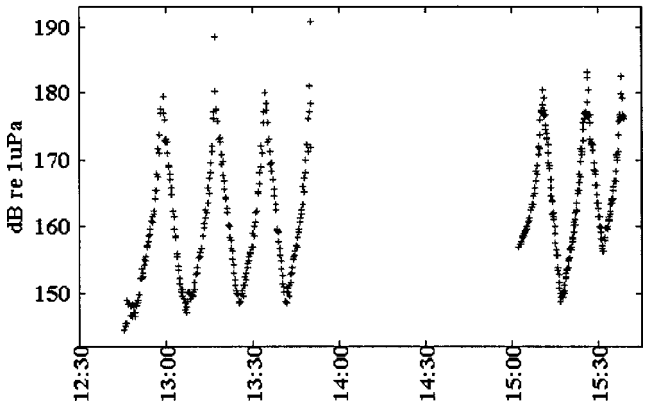

FIG. 1. Received air-gun signal levels with time (lower axis). Units are *mean squared pressure* over the portion of the signal which encompassed 95% of its energy, as measured from the cage bottom. The signal source was moved toward and then away from the cage as described in the text.

four approaches towards the cage over 1:05 h, a break of 1:12 h, and then three further approaches over 0:36 h, as shown in Fig 1. Group III fish held after exposure continued to grow and showed no signs of disease.

Trials were carried out in Jervoise Bay, Western Australia. The average depth of the bay is 9 m. Captive fish were held in a 10-m-long by 6-m-wide by 3-m-deep cage or in 1-m^3 cages. A 0.33 L (20 cubic inch) Bolt PAR 600B air-gun deployed at 5-m depth and operating at a gas pressure of 10 MPa and a 10-s repetition period (6 pulses per minute) was towed from start up at 400–800 m away to 5–15 m at closest approach to the cage. This air-gun has a source level at 1 m of 222.6 dB *re* 1 μPa peak to peak, or 203.6 dB *re* 1 μPa mean squared pressure. Example power spectra of the air-gun signal received at the cage from 50 and 100 m are shown in Fig. 2. The frequency spectra of the air-gun signal had highest energy over 20–100 Hz (the bubble pulse energy) and significant energy over the 100–1000 Hz range. The air-gun signal at 100-m range was more than 25 dB above the background level in the bay, over 100–1000 Hz. Most fish are known to have their best sensitivity to sound energy in the frequency range 100–1000 Hz (Fay, 1988), although many fish also display high sensitivity at lower frequencies (Sand and Karlsen, 1986).

Hydrophones deployed on the long axis of the cage, facing the closest air-gun passage and at depths of 0.2 and 3 m,

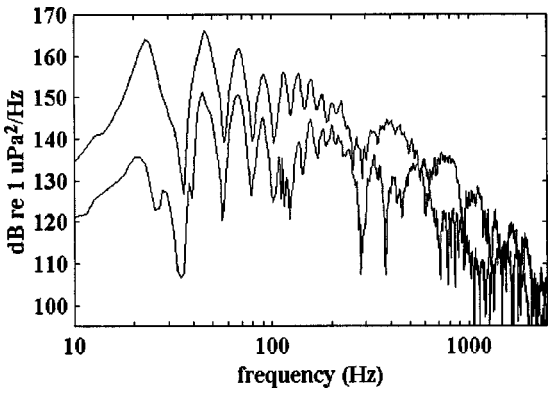

FIG. 2. Power spectra of air-gun signals received at the cage, at 50 m (top curve) and 100 m (bottom curve) range (1.27-Hz frequency resolution).

were used to record air-gun signals. Two underwater video cameras placed in corners of the cage were used to monitor fish behavior. Pink snapper were obtained from an aquaculture farm and acclimated for 24 days at the trial site. Mean fish lengths the day of exposure were 230±24 mm and 58 days later 250±8 mm.

At appropriate times, fish were sacrificed with an overdose of 2-phenoxyethanol. The cranium was quickly opened, and the ears were exposed and fixed in 4% gluteraldehyde buffered with filtered seawater. Ears were dissected out, dehydrated, and progressively graded through 50%, 60%, 70%, 90%, 95%, and 100% acetone, critically point dried, mounted on stubs and sputter coated with gold (2 min).

Tissue was viewed with a Philips XL 30 SEM. To quantify damage, three vertical transects of digital images (each 532×712 pixels) were made along the horizontally aligned epithelium—near the caudal, midsection, and rostral ends [Fig. 3(a)]. Vertically adjacent images (800× magnification) were taken down each transect. For each correctly scaled image, overlain with 25-μm gridlines, the number of missing hair cells in 24×625 μm^2 squares were counted. Missing hair cells were obvious as a ''rounded hole'' in the epithelia in an expected hair cell position. ''Expected'' hair cell locations were based on the local matrix of hair cells. Any 24 ×625 μm^2 square which had an artifact of some sort or which did not encompass a full field of hair cells was not included in counts and 625 μm^2 were subtracted from the total area searched per image. Artifacts included preparation tears, overexposed regions of image or regions covered in ''gunk'' which obscured hair cells and epithelia. Only sections of epithelia populated with hair cells were included in calculations (i.e., edges were not included). Counts were conservative. Thus, any holes of which we were not certain were not included. Experiments were carried out under Curtin University Animal Experimentation Ethics permits.

III. RESULTS

The sensory hair cells of fish ears are similar to those of other vertebrates (Popper and Fay, 1999). The fish ear acts as an accelerometer, with hair cell deflection driven by differential motion between a dense calcareous otolith and a sensory epithelium (deVries, 1950; Popper and Fay, 1999). Using scanning electron microscopy, we analyzed hair cells on the sensory epithelium of the saccule [Fig. 3(a)], the otolithic end organ primarily involved in hearing in most fish species (Popper and Fay, 1999). The epithelia of group I (control) snapper had an appearance similar to that reported for other species of fishes (Popper and Fay, 1999), with fields of ciliary bundles distributed across the epithelia [Figs. 3(b) and (c)]. A small number of holes, correlating with the expected locations of hair cells, were found in the group I epithelia.

Group II fish (sacrificed 18 h after exposure) were observed to have localized dense patches of holes and ''blebbing'' or ''blistering'' on the surface of the epithelia coincident with the location of hair cells [Fig. 4(c)]. However, when the number of holes/10 000 μm^2 along three transects across the epithelium was compared with the group I fish (controls) [Table I, Fig. 3(a)], group II fish did not have

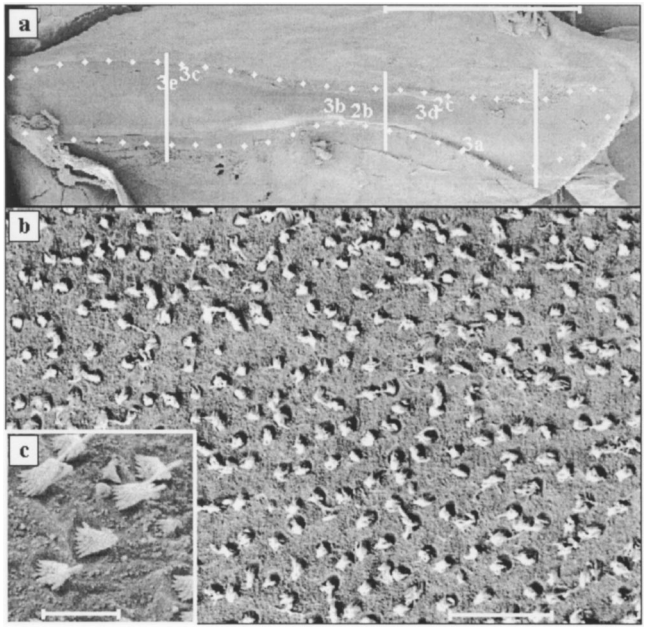

FIG. 3. (a) Horizontally aligned, sensory epithelium from a right pink snapper ear (anterior to the left, dorsal to the top). The area containing sensory hair cells is shown bounded by white dots. The locations of the three transects taken on each epithelium are shown by the white vertical lines. The locations of the various images used in this figure and in Fig. 4 are indicated with the figure number. (b, c) Undamaged hair cells from group I fish. (b) A field of normal ciliary bundles on the sensory hair cells. (c) Higher magnification of several ciliary bundles. Scale bars: (a) 2 mm; (b) 20 μm; (c) 2 μm.

significantly greater numbers of holes/10 000 μm^2 than group I fish ($p > 0.1$, two tailed *t*-test).

In contrast, group III specimens [sacrificed 58 days after exposure, Figs. 4(d) and (e)] showed significantly greater numbers of holes/10 000 μm^2 than group I or II fish ($p \ll 0.001$, two tailed *t*-test), and greater areas of ''blebbing.'' The nature of the holes suggest that hair cells had been ''ripped'' from the epithelia (immediate mechanical damage) or, alternatively, had ''exploded'' after exposure (physiological damage associated with cell death, group III fish). ''Blebbing'' was consistent with expansions of the hair cell ciliary bundle surface, with eventual rupture leading to a hole [Fig. 4(e)]. The finding of significantly more damage in group III fish compared to group I or II is consistent with previous findings that have shown damage to hair cells is not visible until one or more days after the exposure to intense noise (Corwin and Cotanche, 1988; Hastings *et al.*, 1996).

TABLE I. Number of holes in the epithelia of sample groups.

Group[a]	N (E)[b]	N (I)[c]	Holes[d]	Area (10 000 μm^2)[e]	Holes/10 000 μm^{2f}
I	6	84	58	119.19	0.53±0.227
II	3	38	39	54.75	0.75±0.455
III	5	56	665	76.69	8.48±2.636

[a]Exposure regimen.
[b]Number of epithelia examined.
[c]Number of images analyzed per group.
[d]Total number of holes per group.
[e]Total area perused per group.
[f]The mean ±95% confidence limits of the ratio of holes per area, using all images per group (not the same as total holes divided by total area per group).

To give an indication of the relative level of damage as indicated by the presence of missing hair cells, we compared the number of holes with hair cell densities. To obtain an estimate of hair cell density was the total number of hair cells counted in 97 625-μm^2 grids across five images from three epithelia, one a control group I fish and two from group II fish. The mean density of hair cells across the three epithelia was 317±8.9 hair cells/10 000 μm^2 ($n = 97 \times 625$ μm^2 squares, ±95% confidence limits). The tight confidence limits about the mean value implied that hair cell densities were consistent across epithelia. Thus, using this value as a general estimate of hair cell density across the epithelia, localized damage reached 15% (hair cells missing) at the caudal end of the saccular epithelium of group III fishes. Although when averaged across the three transects,

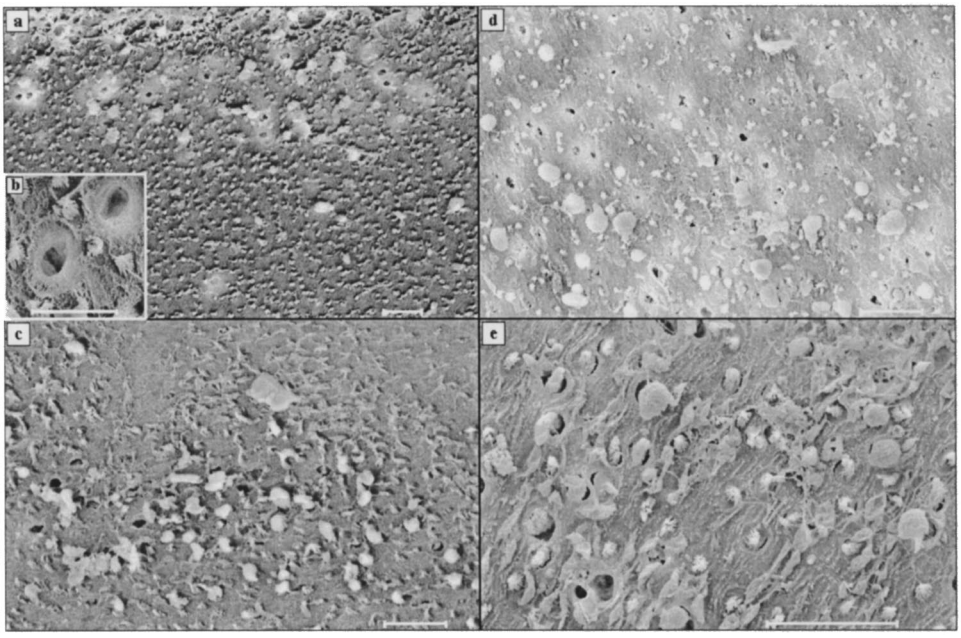

FIG. 4. (a, b, c) Epithelia from group II fish (18 h after exposure to the airgun). The photographs show numerous holes and ''blebbing.'' (d, e) Photographs from saccular epithelia of group III fish (58 days after exposure) tissue showing extensive damage. Scale bars: (a) 20 μm; (b) 2 μm; (c, d, e) 20 μm.

the number of missing cells/10 000 μm^2 for the group III fishes was relatively low (2.7%), it should be noted that the counts were of gross damage only as evidenced by hair cells ejected from the epithelia. Damage severe enough to create the holes must have had wider implications for remaining hair cells, particularly for tip link function (Pickles, 1993). Without neurophysiological experimentation, it was not possible to determine if hair cells remaining intact on the epithelia after air-gun exposure were still fully functional.

IV. DISCUSSION AND CONCLUSIONS

It is known that a number of species of teleost fish continue to produce sensory hair cells for much of their lives (Popper and Hoxter, 1994; Lombarte and Popper, 1994) and that sensory hair cells are regenerated after insult with ototoxic drugs in at least one species, and probably all (Lombarte *et al.*, 1993). It is also known that some avian species will regenerate sensory cells after intense sonic insult (Corwin and Cotanche, 1988). However, the damage in the ears of the pink snapper suggests that regeneration, even if it occurred over 58 days, did not counteract the loss of cells resulting from sonic insult. Either damage continued to accrue well after insult, regeneration was slowed or ceased, or significant regeneration did not occur until beyond the 58-day sample period.

There are a number of caveats that must be pointed out in considering the implications of our results. First, the fish studied were caged and could not swim away from the sound source. Video monitoring of behavior suggested that the fish would have fled the sound source if possible. It is also likely that many, but perhaps not all, species hearing an approaching air-gun would swim away, as has been observed on a large scale by Engås *et al.* (1996). Second, we only examined a single species. While the snapper ear is typical of the majority of commercially important species (e.g., salmon, tuna, cod, haddock) (Dale, 1976; Popper, 1977; Lombarte and Popper, 1994), it is possible that pink snapper are more or less sensitive to intense stimulation than other species. Third, the impact of exposure on ultimate survival of the fish is not clear. Behavioral studies have observed that some fish exposed to air-gun signals display aberrant and disoriented swimming behavior, suggesting that damage to the ears may also have vestibular impact (authors, personal observation). Fishes with impaired hearing would have reduced fitness, potentially leaving them vulnerable to predators, possibly unable to locate prey, sense their acoustic environment, or, in the case of vocal fishes, unable to communicate acoustically. Fourth, although the full exposure regimen was accurately quantified, the approach-departure nature of trials meant a precise air-gun exposure required to produce the damage observed was not obtained. Was it the few high level signals or the accumulation of many moderate to high level signals? The sound exposure (intensity and time) required to produce damage has important ramifications in the range from a full scale seismic source at which such impacts may be expected. As a comparison, air-gun signals of level $\geqslant$180 dB *re* 1μPa (*mean squared pressure*, see Fig. 1 for levels experienced by fish in these trials) could be expected at ranges $<$500 m from a large seismic array (44 L, R.M. data).

This study demonstrates that exposure to seismic air-guns can cause significant damage to the ears of fishes. While additional studies are needed to better understand the mechanical and physiological process leading to damage, the repair process, impact on behavior and fitness, and the exposure regimen required to produce a specified amount of damage, our results suggest caution in the application of very intense sounds in environments inhabited by fish. Furthermore, given that hair cells form the ultimate end organs of the hearings system of all vertebrates, the results presented here may have important implications for other marine vertebrates.

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