

Intense ultrasonic clicks from echolocating toothed whales do not elicit anti-predator responses or debilitate the squid *Loligo pealeii*

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Toothed whales use intense ultrasonic clicks to echolocate prey and it has been hypothesized that they also acoustically debilitate their prey with these intense sound pulses to facilitate capture. Cephalopods are an important food source for toothed whales, and there has probably been an evolutionary selection pressure on cephalopods to develop a mechanism for detecting and evading sound-emitting toothed whale predators. Ultrasonic detection has evolved in some insects to avoid echolocating bats, and it can be hypothesized that cephalopods might have evolved similar ultrasound detection as an anti-predation measure. We test this hypothesis in the squid *Loligo pealeii* in a playback experiment using intense echolocation clicks from two squid-eating toothed whale species. Twelve squid were exposed to clicks at two repetition rates (16 and 125 clicks per second) with received sound pressure levels of 199–226 dB re 1 μ Pa (pp) mimicking the sound exposure from an echolocating toothed whale as it approaches and captures prey. We demonstrate that intense ultrasonic clicks do not elicit any detectable anti-predator behaviour in *L. pealeii* and that clicks with received levels up to 226 dB re 1 μ Pa (pp) do not acoustically debilitate this cephalopod species.

Keywords: squid; toothed whales; echolocation; predation; defence

1. INTRODUCTION

Toothed whales play an important role as top predators in aquatic ecosystems (Clarke 1977). Cephalopods are part of the diet of 60 out of 67 toothed whale species and constitute the main food source of at least 28 species (Clarke 1996). The large cephalopod biomass consumed by toothed whales (Clarke 1977) suggests that cephalopods would be exposed to a considerable selection pressure to evolve a way of detecting and evading echolocating predators. Most toothed whales ensonify their prey by emitting short ultrasonic clicks

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with source sound pressure levels around 220 dB re 1 μ Pa (pp) (Au 1993), which has been hypothesized to acoustically debilitate their prey (Norris & Møhl 1983). Cephalopods and other toothed whale prey species are faced with a predator that, for prey species that can detect ultrasound, loudly announces its approach many times per second. Therefore, it can be hypothesized that ultrasonic clicks would be efficient cues for cephalopod prey to reduce predation pressure by initiating anti-predator behaviours.

This kind of acoustic interaction between an echolocating predator and prey is found in bats and some insect species that have developed ears sensitive to the ultrasonic echolocation signals of bats. When these insects detect the ultrasonic bat cries, they initiate a series of strong anti-predator responses (Miller & Surlykke 2001). While these aerial acoustic predator–prey interactions are well documented for bats and some insect species, ultrasound detection has been found only in a few fish species (Popper *et al.* 2004), and it has never been tested in cephalopods. Sound detection in cephalopods has been investigated in the frequency range below 1 kHz and cephalopods are reported to detect the particle motion component of the sound field in the low frequency range from one to a few hundred hertz (Hanlon & Budelmann 1987; Packard *et al.* 1990). Here, we test the hypotheses that intense toothed whale echolocation clicks can debilitate cephalopods, making them easier to capture, and that cephalopods have evolved a capability to detect intense echolocation clicks from toothed whales and respond to them by initiating anti-predator behaviours.

2. MATERIAL AND METHODS

We used the squid *Loligo pealeii* that is heavily preyed upon by toothed whales on the continental shelves (Clarke 1996) and for which an established ethogram exists (Hanlon *et al.* 1999). Twelve adult *L. pealeii* (two males and 10 females) were caught off Woods Hole and housed at the Marine Biology Laboratory.

Six trials with two squid in each were conducted in a circular test tank (figure 1). Each squid was used in a single trial consisting of eight exposures with 2 min in between. For each trial, the squid were exposed to two sound types: a broadband delphinid echolocation click and a frequency-modulated click from a beaked whale played at two repetition rates of 16 and 125 clicks per second (electronic supplementary material). The two echolocation signals were synthesized on the basis of field recordings of beaked whales and dolphins (Au 1993; Madsen *et al.* 2005). The squid were exposed to each click train twice. The two repetition rates were chosen to mimic the fast search mode of an echolocating toothed whale (16 clicks per second) and the buzz phase just prior to prey capture where toothed whales produce clicks at high repetition rates (125 clicks per second) (Madsen *et al.* 2005). In the exposure zone, the squid received sound pressure levels of 202–209 dB re 1 μ Pa (pp) (energy flux density: 179–188 dB re 1 μ Pa² s) when the beaked whale click train was used and 199–207 dB re 1 μ Pa (pp) (energy flux density: 183–193 dB re 1 μ Pa² s) when the delphinid click train was used, corresponding to received sound pressure levels 10 m from echolocating toothed whales using maximum output or the levels received at close range from a toothed whale in the buzz/capture phase (Madsen *et al.* 2005).

Squid are known to elicit a characteristic set of anti-predator behaviours such as the blanch-ink-jet manoeuvre, and we evoked this behaviour after each trial as a baseline by moving a landing net rapidly through the water close to the squid. Cephalopods use various forms of visual crypsis as their primary defence against predators (Hanlon & Messenger 1996). When crypsis fails, and cephalopods are being approached, they adopt one of two tactics: go or stay. When they choose to go, they execute a rapid characteristic behaviour called blanch-ink-jet manoeuvre (Hanlon & Messenger 1996). The cephalopod blanches white or dark and ejects ink as it jets away, leaving ink as a pseudomorph decoy in its place. With the stay tactic, the animals show some form of deimatic

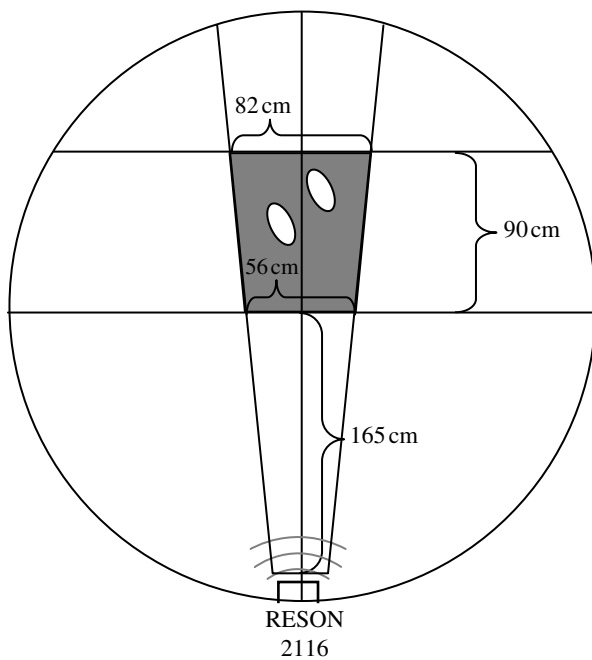


Figure 1. Test tank with strings marking the beam cone of the transducer. The squid (white ovals) were stimulated only when inside the grey-shaded exposure zone to ensure sound pressure levels corresponding to the predators. See text.

behaviour that is characterized by conspicuous body patterns and postures (figure 2a). These behaviours and their criteria in *L. pealeii* have been described in detail (e.g. Hanlon *et al.* 1999) and to quantify these behavioural responses and test for their presence, video recordings of the squid were analysed by comparing a 3 s pre-exposure and the 3 s exposure scoring for the following behaviours: (i) change in chromatophore patterning of the skin, (ii) inking present, and (iii) fast jetting present.

To test the acoustic debilitation hypothesis, we conducted a second experiment in which three squid were placed in a smaller test tank. They were stimulated with the same clicks as before, but only when they passed closely in front of the transducer (0.3–0.6 m) with received sound pressure levels of 222–226 dB re 1 μPa (pp) (figure 2b).

3. RESULTS

Pre-stimulation behaviour of the squid consisted of stereotyped hovering slowly back and forth over the bottom. When stimulated, the squid were exposed to sound pressure levels of 199–209 dB re 1 μPa (pp) depending on their position in the exposure zone. Careful examination of the video recordings revealed no changes in chromatophore patterning in the skin, no fast jetting, inking or moving away from the transducer irrespective of click type and repetition rate. Thus, following the criteria listed above, the exposed *Loligo* did not show any apparent behavioural responses to the four different exposures of intense ultrasound (table 1). The second experiment of high sound pressure level exposures did not elicit any apparent reactions either, as the squid kept swimming back and forth in close proximity to the transducer, while displaying normal behaviour (figure 2b).

In contrast to the sound exposures, all the squid showed a strong and distinct anti-predator response to the control exposure of a fast-moving landing net (table 1).

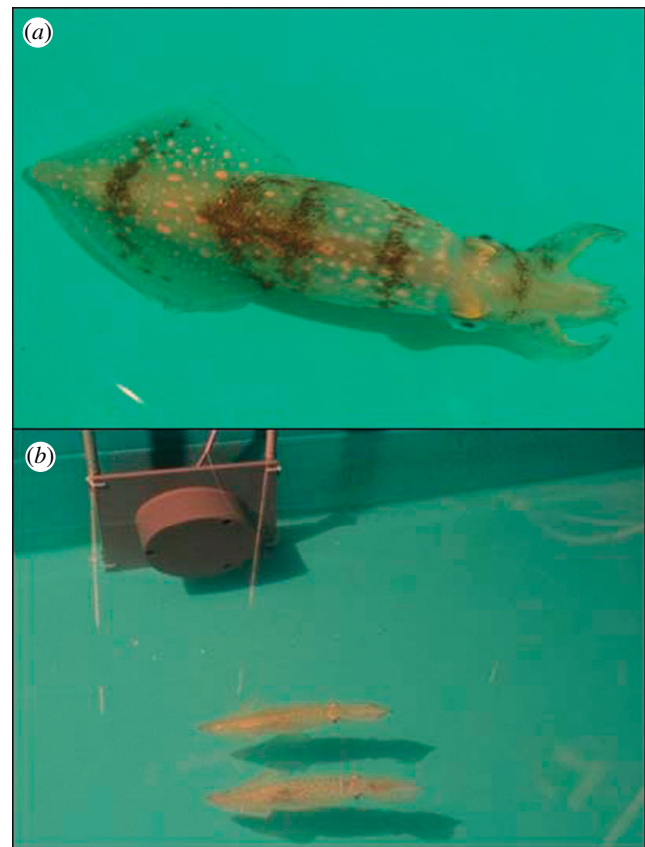


Figure 2. (a) *Loligo pealeii* showing a typical response to threat by settling at the bottom and exhibiting disruptive coloration characterized by dark transverse bands on the body. (b) Two *L. pealeii* passing in front of the transducer. The squid are receiving clicks with sound pressure levels of 223 dB re 1 μPa (pp) but continue normal swimming and coloration.

4. DISCUSSION

Given that predation by toothed whales exerts a selection pressure on cephalopods (Clarke 1977; Moynihan 1985), it is tempting to predict that squid have coevolved the capability to detect the ultrasonic clicks of approaching toothed whales, thereby lowering their risk of predation. But there were no signs of any of the anti-predator behaviours defined from established ethograms of this species (Hanlon *et al.* 1999) that would be expected if the squids detected and perceived the ultrasonic sound pulses as coming from an approaching predator (Hanlon & Messenger 1996; Hanlon & Shashar 2003). This finding in squid is contrary to the strong responses to intense ultrasound of some fish species at much lower received sound pressure levels (Popper *et al.* 2004), and to the responses of some insects to ultrasonic bat signals (Miller & Surlykke 2001). The consistent lack of evidence in the present study that the squid *L. pealeii* can detect and respond to intense echolocation signals from some of its main predators, implies that toothed whales, when foraging for squid, can use ultrasound without alerting the squid acoustically. The acoustic behaviour of echolocating toothed whales and the properties of the sonar signals in use therefore, probably evolved to maximize the success of locating and intercepting squid prey apparently

Table 1. Results of the experiments. (*n*, number of squid; BW, beaked whale echolocation click train; and D, delphinid click train. 125, 125 clicks per second; 16, 16 clicks per second; control, fast-moving landing net.)

exposure	jetting		change in chromatophore expression		inking	
	present	absent	present	absent	present	absent
BW 125 (<i>n</i> =12)	0/12	12/12	0/12	12/12	0/12	12/12
BW 16 (<i>n</i> =12)	0/12	12/12	0/12	12/12	0/12	12/12
D 125 (<i>n</i> =12)	0/12	12/12	0/12	12/12	0/12	12/12
D 16 (<i>n</i> =12)	0/12	12/12	0/12	12/12	0/12	12/12
control (<i>n</i> =11)	11/11	0/11	11/11	0/11	7/11	4/11

without the evolutionary shaping factors of the acoustic 'arms race' observed between echolocating bats and insects with acute ultrasonic hearing.

Norris & Møhl (1983) proposed that toothed whales emit echolocation signals so powerful that the prey is debilitated by the very high sound pressure levels, making capture easier. This idea provoked lively discussion among cephalopod researchers (Moynihan 1985; Hanlon & Budelmann 1987; Budelmann & Bleckmann 1988). In the present experiment, we isolated the acoustic component of the interaction between squid and toothed whales and exposed squid to sound pressure levels of 199–226 dB re 1 μ Pa (pp), which is comparable to the most intense echolocation clicks a squid will be exposed to even from sperm whales. Source sound pressure levels of sperm whale clicks have been measured up to 240 dB re 1 μ Pa (pp) (Møhl *et al.* 2003), but as sperm whales close in on their prey, they initiate a buzz phase and the sound pressure levels drop some 20 dB or more, as in beaked whales (Madsen *et al.* 2005). Despite the high-intensity levels used here, there were no signs of debilitation or disorientation of the squid at either repetition rate. Thus, high sound pressure levels in toothed whale echolocation systems do not operate to debilitate cephalopod prey, but to maximize biosonar detection of prey targets with weak backscattering properties. This finding is in agreement with the study of Mackay & Pegg (1988), in which a single octopus was stimulated with a 22 kV underwater spark generating an unknown, but likely broadband, high-pressure transient waveform with no signs of acoustic debilitation.

The interactions between predator and prey involve an array of passive and active sensory and behavioural means evolved in an open-ended arms race, balancing the costs of foraging, reproduction and predator avoidance. Here, we have isolated an active primary sensory cue used by echolocation toothed whales to find prey and shown that cephalopods do not respond to intense sound pulses, and that they must use other sensory cues and behavioural measures to lower the predation pressure from large air-breathing predators that have hunted them for more than 20 Myr.

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- Au, W. W. L. 1993 *Sonar of dolphins*. New York, NY: Springer.
- Budelmann, B. U. & Bleckmann, H. 1988 A lateral line analogue in cephalopods: water waves generate microphonic potentials in the epidermal head lines of *Sepia* and *Lolliguncula*. *J. Comp. Physiol. A* **164**, 1–5. (doi:10.1007/BF00612711)
- Clarke, M. R. 1977 Beaks, nets and numbers. *Symp. Zool. Soc. Lond.* **38**, 89–126.
- Clarke, M. R. 1996 Cephalopods as prey. III. Cetaceans. *Phil. Trans. R. Soc. B* **351**, 1053–1065. (doi:10.1098/rstb.1996.0093)
- Hanlon, R. T. & Budelmann, B. U. 1987 Why cephalopods are probably not "deaf". *Am. Nat.* **129**, 312–317. (doi:10.1086/284637)
- Hanlon, R. T. & Messenger, J. B. 1996 *Cephalopod behaviour*. Cambridge, UK: Cambridge University Press.
- Hanlon, R. T. & Shashar, N. 2003 Aspects of the sensory ecology of cephalopods. In *Sensory processing in the aquatic environment* (eds S. P. Collin & N. J. Marshall), pp. 266–282. Berlin, Germany: Springer.
- Hanlon, R. T., Maxwell, M. R., Shashar, N., Loew, E. R. & Boyle, K. L. 1999 An ethogram of body pattering behavior in the biomedically and commercially valuable squid *Loligo pealeii* off Cape Cod, Massachusetts. *Biol. Bull.* **197**, 49–62. (doi:10.2307/1542996)
- Mackay, S. R. & Pegg, J. 1988 Debilitation of prey by intense sounds. *Mar. Mamm. Sci.* **4**, 356–359. (doi:10.1111/j.1748-7692.1988.tb00543.x)
- Madsen, P. T., Johnson, M., Aguilar de Soto, N., Zimmer, W. M. X. & Tyack, P. 2005 Biosonar performance of foraging beaked whales (*Mesoplodon densirostris*). *J. Exp. Biol.* **208**, 181–194. (doi:10.1242/jeb.01327)
- Miller, L. A. & Surlykke, A. 2001 How some insects detect and avoid being eaten by bats: tactics and countertactics of prey and predator. *Bioscience* **51**, 570–581. (doi:10.1641/0006-3568(2001)051[0570:HSIDAA]2.0.CO;2)
- Møhl, B., Wahlberg, M., Madsen, P. T., Heerfordt, A. & Lund, A. 2003 The monopulsed nature of sperm whale clicks. *J. Acoust. Soc. Am.* **114**, 1143–1154.
- Moynihan, M. 1985 Why are cephalopods deaf? *Am. Nat.* **125**, 465–469. (doi:10.1086/284353)
- Norris, K. S. & Møhl, B. 1983 Can odontocetes debilitate prey with sound? *Am. Nat.* **122**, 85–104. (doi:10.1086/284120)
- Packard, A., Karlsen, H. E. & Sand, O. 1990 Low frequency hearing in cephalopods. *J. Comp. Physiol. A* **166**, 501–505. (doi:10.1007/BF00192020)
- Popper, A. N., Plachta, D. T. T., Mann, D. A. & Higgs, D. 2004 Response of clupeid fish to ultrasound: a review. *J. Mar. Sci.* **61**, 1057–1061.