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RESEARCH ARTICLE

Directional escape behavior in allis shad (*Alosa alosa*) exposed to ultrasonic clicks mimicking an approaching toothed whale

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SUMMARY

Toothed whales emit high-powered ultrasonic clicks to echolocate a wide range of prey. It may be hypothesized that some of their prey species have evolved capabilities to detect and respond to such ultrasonic pulses in a way that reduces predation, akin to the situation for many nocturnal insects and echolocating bats. Using high-speed film recordings and controlled exposures, we obtained behavioural evidence that simulated toothed whale biosonar clicks elicit highly directional anti-predator responses in an ultrasound-sensitive allis shad (*Alosa alosa*). Ten shad were exposed to 192 dB re. 1 μPa (pp) clicks centred at 40 kHz at repetition rates of 1, 20, 50 and 250 clicks s^{-1} with summed energy flux density levels of 148, 161, 165 and 172 dB re. 1 μPa^2 s. The exposures mimicked the acoustic exposure from a delphinid toothed whale in different phases of prey search and capture. The response times of allis shad were faster for higher repetition rates of clicks with the same sound pressure level. None of the fish responded to a single click, but had median response times of 182, 93 and 57 ms when exposed to click rates of 20, 50 and 250 clicks s^{-1}, respectively. This suggests that the ultrasound detector of allis shad is an energy detector and that shad respond faster when exposed to a nearby fast-clicking toothed whale than to a slow-clicking toothed whale far away. The findings are thus consistent with the hypothesis that shad ultrasound detection is used for reducing predation from echolocating toothed whales.

Key words: ultrasound, detection, directional response, allis shad, toothed whale.

INTRODUCTION

Echolocating toothed whales are important predators in the marine environment with an annual marine biomass turnover surpassing the annual landings by the world's fishing fleets (Clarke, 1977). To orient and to locate food, they emit short, highly directional ultrasonic clicks in the frequency range of 15–180 kHz (Au, 1993; Møhl and Andersen, 1973), with source sound pressure levels between 140 and 240 dB re. 1 μPa (peak to peak; pp) at 1 m (Au, 1993; Møhl et al., 2003). Until now, however, few studies have been done to test how predation by echolocating toothed whales has affected the evolution of sensory modalities and avoidance reactions in their prey organisms.

More is known about the acoustic interaction between bats and their prey. Like toothed whales, bats emit intense ultrasonic cries and use the echoes reflected from objects to guide motor patterns during search for and capture of their prey (Griffin, 1958). It is generally accepted that the heavy predation pressure from echolocating bats has led to convergent evolution of ears sensitive to the ultrasonic biosonar signals of bats in several distantly related families of moths (Miller and Surlykke, 2001) as well as in a number of other nocturnal insects (Hoy and Robert, 1996; Yack and Fullard, 1993). When these insects are exposed to ultrasonic cries from bats, they exhibit a complex pattern of responses depending on the repetition rate as well as the intensity of the echolocation signals impinging on them (Miller and Surlykke, 2001; Roeder, 1964). It may be hypothesized that the predation pressure from echolocating toothed whales might have driven a similar evolution of ultrasound detection in some of the species of fish and cephalopods they prey upon (Astrup, 1999; Mann et al., 1998).

Many squid and fish species are important food sources for toothed whales (Santos et al., 2001), but only a few studies have addressed whether these species can detect echolocating toothed whales. Longfin squid (*Loligo pealeii*) do not show any detectable response when exposed to very intense ultrasound (Wilson et al., 2007), and most fish species studied so far can only detect sounds up to a few kHz (Hawkins, 1981). A study by Astrup and Møhl reported that cod could be conditioned to respond to 38 kHz ultrasonic pulses with a very high detection threshold of 203 dB re. 1 μPa (pp) and proceeded to speculate that ultrasound detection in cod is caused by overstimulation of skin receptors (Astrup and Møhl, 1993). However, unconditioned cod do not respond to intense ultrasound (Schack et al., 2008) and are therefore unlikely to use ultrasound detection as a way to reduce predation by echolocating toothed whales (Astrup and Møhl, 1993; Schack et al., 2008). Subsequently, a few herring species belonging to the subfamily Alosinae (shads and menhaden) have been shown to respond to ultrasound in the frequency range of clicks from echolocating toothed whales at much lower sound pressure levels than the cod (Dunning et al., 1992; Mann et al., 2001; Mann et al., 1997; Plachta and Popper, 2003; Wilson et al., 2008). As opposed to Astrup's skin receptor detection hypothesis, it has been suggested that the inner ear is the ultrasound detection organ (Mann et al.,

1998; Higgs et al., 2004), but recent findings by Wilson et al. have shown that the lateral line is involved in ultrasound detection (Wilson et al., 2009).

If the ultrasound sensitivity of Alosinae has evolved to serve detection and avoidance of echolocating toothed whales, it requires that the fish, when exposed, exhibit an evasive reaction increasing the chance of survival (Endler, 1986). More specifically, it can be hypothesized that the fish must be able to assess direction and proximity of the toothed whale predator (Astrup and Møhl, 1997; Miller and Surlykke, 2001). Previous playback studies have shown that shad exposed to pure tone ultrasound played at varying sound pressure levels show a graded directional response: if the sound is very intense the fish will exhibit a very strong and panic-like response, whereas a lower sound pressure level yield a weaker response (Plachta and Popper, 2003; Wilson et al., 2008). Another possible cue to the proximity of the predator is the rate at which a toothed whale is clicking (Astrup, 1999; Astrup and Møhl, 1997). Like echolocating bats, toothed whales produce echolocation clicks at a higher repetition rate when they approach their prey, and most prey capture attempts are terminated with a buzz phase during which the repetition rate is up to several hundred clicks per second (Miller et al., 1995; Madsen et al., 2002; Madsen et al., 2005; Deruiter et al., 2009; Verfuss et al., 2009). Thus, if ultrasound detection in Alosinae is used as a way to avoid predation from toothed whales, we hypothesize that Alosinae will exhibit negative phonotaxis, i.e. a directional evasive manoeuvre increasing the distance to the toothed whale when exposed to ultrasonic clicks. We further predict that like some moth species, they will exhibit different response patterns depending on the proximity of the toothed whale (Plachta and Popper, 2003), as indicated by the repetition rate of the ultrasonic biosonar clicks.

In this study we quantified behavioural reactions of allis shad (*Alosa alosa*) exposed to directional ultrasonic clicks to assess whether the behavioural responses are likely to increase the chance of survival when encountering an echolocating toothed whale.

MATERIALS AND METHODS

Experimental setup and animals

The experiments were conducted in May 2007 and 2008 at the INRA Station de piégeage, Le Moulin des Princes, Pont Scorff in Le Scorff River (Brittany, France). Adult *Alosa alosa* L. were caught in a fish trap at the station during their upstream migration toward their spawning grounds. Seventeen fish of mixed sex with body lengths between 45 and 55 cm were used, but some of the fish did not cope well with being in the test tank and were excluded from the experiment and released back into the river. Data was collected from ten fish that were released in the river upstream from the trap after the experiments were completed. The experiment took place at the station in a covered outdoor square PVC test tank measuring 2.1×2.1×0.37 m (length × width × depth). We covered the test tank to prevent any visual stimuli interfering with the experiment. The test tank was filled with water from the river, at a temperature of 13°C. A square (0.25×0.3 m) in the middle of the bottom of the test tank marked the acoustic exposure zone 0.75 m from the transducer (Fig. 1).

Sound exposure

We designed a 0.2 ms click consisting of six cycles at 40 kHz (Fig. 2A,B). Clicks were transmitted using a directional Reson 2116 transducer (transmitting sensitivity of 172 dB re. 1 µPa V^{-1} at 1 m, half power beam width of 13 deg) connected to an arbitrary waveform generator (Agilent 33220A). To mimic the acoustic exposures from toothed whales at different ranges, we played simulated echolocation click trains with a total duration of 1 s at four different repetition rates of 1, 20, 50 and 250 clicks s^{-1} (Fig. 2C). Approximately 250 clicks s^{-1} are emitted from at toothed whale in the final prey capture phase, when it is within a few metres of the prey (Miller et al., 1995; Madsen et al., 2005; Deruiter et al., 2009). Repetition rates of 50 clicks s^{-1} and 20 clicks s^{-1} mimic a delphinid toothed whale in the approach phase during a prey capture when it would be approximately 10–30 m from the prey (Au, 1993) and a single click could be from scanning toothed whales, that has not locked the biosonar on the prey.

Among the toothed whales, members of the Delphinidae family are the ones that would be most likely to feed on shads. Several species of delphinids seem to adjust their sonar outputs to keep a level around 180–195 dB re. 1 µPa (pp) on their target while approaching it (Au and Benoit-Bird, 2003; Jensen et al., 2009). During all experiments performed, the sound pressure level was therefore kept at 192 dB re. 1 µPa (pp) (±3 dB) in the exposure zone. The energy flux density (EFD) of each click was 148 dB re. 1 µPa^2 s in the exposure zone and for the three repetition rates of 20, 50 and 250 clicks s^{-1}, the maximum received EFD levels were 161, 165 and 172 dB re. 1 µPa^2 s, respectively, during the 1 s exposure (Fig. 2D). Owing to a technical problem, some exposures at 20 clicks s^{-1} contained a few erroneous sound pulses (visible in the second trace of Fig. 2C), but the reported sound exposure levels are computed from measurements in the tank and will therefore include the contributions from the extra erroneous pulses (Fig. 2D). At 1, 50 and 250 clicks s^{-1} there were no erroneous sound pulses. We

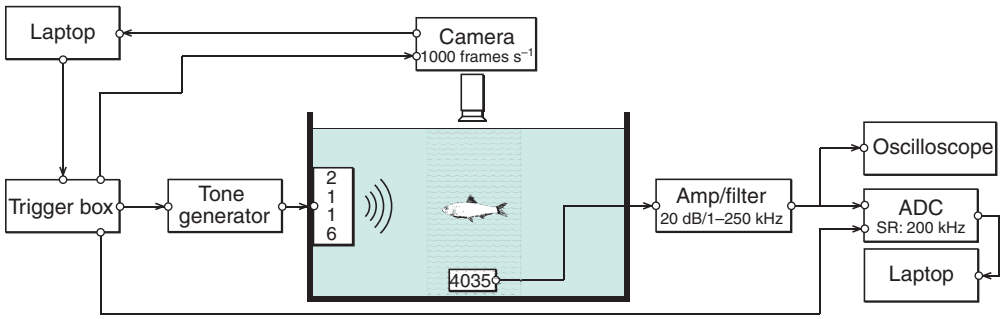

Fig. 1. Experimental setup. The sound exposure zone is shown by wavy grey lines. The swimming behaviour of the fish was observed with a digital high-speed video system connected to a laptop-controlled trigger box. The trigger box was also connected to the sound exposure equipment consisting of a tone generator connected to a transducer Reson 2116 and to the sound recording equipment, consisting of a Reson 4035 hydrophone connected to a amplifier/filter box (20 dB gain; bandpass filter, 1–250 kHz) connected to an analogue-to-digital converter (Wavebook 512).

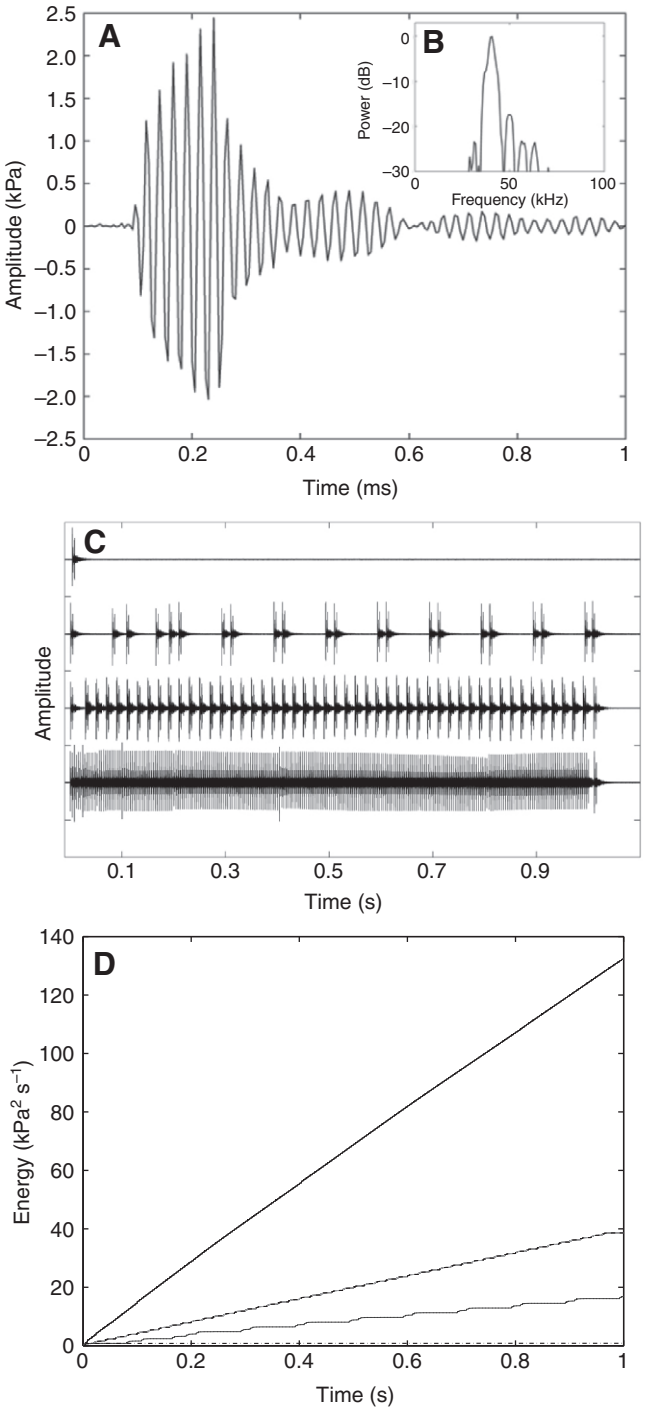

Fig. 2. (A) Waveform and (B) power spectrum of a single ultrasonic click stimuli (sample rate: 200 kHz; bandpass filtered: 10–80 kHz; spectrum computed with a 256-point fast Fourier transformation). (C) Waveforms for four repetition rates of clicks (top to bottom: 1 20, 50 and 250 clicks s^{-1}). (D) Accumulating energy flux density over a 1-s exposure given on a linear scale for each stimulus (solid line: 250 clicks s^{-1}; dashed line: 50 clicks s^{-1}; dotted line: 20 clicks s^{-1}; dashed and dotted line: 1 click s^{-1}).

calibrated the sound field in three dimensions in the entire sound exposure zone using a Reson 4035 hydrophone connected to a Wavebook 512a (Iotech, Inc., Cleveland, OH, USA) sampling at 200 kHz with 12 bit resolution *via* an Reson 6073 amplification/filter box (20 dB gain; bandpass filter 1–250 kHz). Signal analysis of the click trains was performed using custom written scripts in Matlab 6.5 (MathWorks, Natick, MA, USA).

Noise in the test tank was measured with a calibrated Reson 4032 hydrophone. The spectral noise level in the tank was less than 100 dB re. 1 $\mu Pa^2 Hz^{-1}$ between 1 and 50 kHz.

Observation equipment

The swimming behaviour of the fish was recorded with a laptop-controlled, digital high-speed video system (MotionPro HS4 digital camera; Redlake, Virum, Denmark) sampling at 1000 frames s^{-1} and mounted 1.5 m above the water surface of the tank. The camera view did not cover the entire test tank, but a square in the middle of the test tank measuring 60×60 cm. A custom-build trigger system connected to the video system, the sound generator and the sound recording system, synchronized the sound emission, and the video and sound recordings (Fig. 1). The synchronization of sound and the camera pictures was off by less than 5 ms. There was a ring buffer in the audio and video recording systems enabling pre-trigger sampling. Audio and video recordings started 4 s before sound exposure and ended 4 s after triggering.

Experimental approach

The fish were used one at a time for each test. A fish was placed in the test tank 1–3 h prior to the first test session to let the fish habituate to the tank and transducers. A test session consisted of exposure to the four different click trains with a varying repetition rate of the clicks. There was at least 5 min pause between exposures to click trains, and each fish was tested two to four times at each of the click trains, depending on the condition of the fish. Sound emission was started manually when the head of the fish was in the exposure zone as monitored by the experimenter observing the camera input on a laptop screen. The fish was kept in the test tank for a maximum of 5 h.

Reaction time and swimming speed

The onset of response was defined as a C-bend of the body followed by an increase in swimming speed. If there was no sign of C-bend or significant increase in swimming speed, the fish was classified as not responding to the ultrasound. The reaction time for the fish was found by analysing single video frames using Adobe After Effects (version C3, Adobe Systems Inc., San Jose, CA, USA) and Adobe Illustrator (version C3, Adobe Systems Inc.). One vector represented the swimming direction of the fish before exposure. It was defined by the midline of the fish from snout to pectoral fins at time of onset of ultrasound emission. The second vector was defined by the swimming direction of the fish as it changed frame by frame. The reaction time was defined as the time from onset of signal emission to when the second vector of the fish head diverged from the initial vector by 5 deg. Thus the reaction time includes the time it takes for the fish to detect the sound, execute its motor pattern after the sensory threshold is reached combined with the time it takes for the fish to diverge from the initial swimming direction by 5 deg.

To test for difference in swimming speed at different exposures, the swimming speed was analysed in steps of 50 ms before and after sound exposure for the ten fish. An average was estimated for each fish at the four different repetition rates before and after sound exposure, and median swimming speeds for all ten fish is given in Table 1. Swimming speed measurements were made during a 1.5 s interval right before sound exposure and during the first 500 ms after sound exposure (1 click s^{-1}) or after the fish had exhibited a C-bend

Table 1. Reaction time and swimming speed before and after allis shad were exposed to ultrasonic clicks played at repetition rates of 1, 20, 50 and 250 clicks s^{-1} and the received amount of energy when the fish exhibited a startle response

Exposure	1 click s^{-1}	20 clicks s^{-1}	50 clicks s^{-1}	250 clicks s^{-1}
Median reaction time (ms)	–	182 (158; 195)	93 (90; 140)	57 (52; 80)
EFD (dB re 1 μPa^2 s)	–	156 (154; 156)	156 (156; 158)	160 (159; 161)
Median swimming speed (m s^{-1}) pre sound exposure	0.26 (0.20; 0.27)	0.25 (0.20; 0.27)	0.27 (0.2; 0.27)	0.25 (0.2; 0.26)
Median swimming speed (m s^{-1}) post sound exposure	0.25 (0.21; 0.27)	0.32 (0.29; 0.36)	0.43 (0.31; 0.48)	0.55 (0.44; 0.7)

Figures in brackets are the upper and lower quartiles (N=10).
EFD, energy flux density.

(20, 50 and 250 clicks s^{-1}), and the swimming speeds for each interval were calculated. In some sequences (12 out of 60) the fish disappeared out of the view of the camera before the 500 ms of the second interval had passed and therefore in those cases the calculated swimming speed was based on fewer measurements (five to nine).

Statistics

Statistical analyses were performed using PAST (version 1.75b) and Matlab 6.5 (MathWorks). A non-parametric Wilcoxon test for matched pairs was used to test whether the median for each of the treatments was significantly different from the others. According to the standard Bonferroni test, if a P-value is less than or equal to 0.05/n (where n is the number of tests) the hypothesis that the medians were significantly different is accepted (Rice, 1988). The binomial distribution was used to test if there was a tendency for the fish to swim in the direction of 180±30 deg, which is the opposite direction of the sound source. The outcome was considered significant if a P-value was less than or equal to 0.05.

Control experiments

To make sure that the fish responded to the ultrasonic output of the transducer, and not to any omnidirectional low-frequency by-products or electrical impulses from the transducer, three fish were exposed to a low-pass filtered version (cut-off frequency 10 kHz, fourth order) of the 250 Hz click train.

A second control consisted of testing five goldfish, a fish with good hearing in the low frequency range, but no sensitivity to ultrasound (Fay, 1969), with the same equipment, but in a smaller test area (1×1 m). The fish were exposed to 40 kHz clicks played at 250 clicks s^{-1}, with a received sound pressure level of 180 dB re. 1 μPa (±6 dB).

RESULTS

None of the ten tested fish exhibited any detectable response when exposed to single clicks with a received sound pressure level of 192 dB re. 1 μPa (pp) and an EFD of 148 dB re. 1 μPa^2 s. However, they all showed a clear response in the form of a C-bend and increased swimming speed when exposed to trains of ultrasonic clicks played at 20, 50 and 250 clicks s^{-1} with median reaction times of 182 ms [lower quartile (lq) 158, upper quartile (up) 195], 93 ms (lq 90, uq 140) and 57 ms (lq 52, uq 80), respectively (Table 1 and Fig. 3). There was a significant difference in the median reaction times at 250 and 50 clicks s^{-1} (Wilcoxon test, P<0.01, N=10, n=3) and 250 and 20 clicks s^{-1} (Wilcoxon test, P<0.01, N=10), but not at 50 and 20 clicks s^{-1} (Wilcoxon test, P=0.04, N=10).

Based on the click rates and the reaction time (defined as the time at which the fish had diverged by 5 deg from the initial swimming direction at the time of sound exposure), we derived the EFD level that the fish had received at the onset of a response for the different repetition rates (Table 1). There are differences of up to 4 dB in the received EFD at time of response for the three different repetition rates [median: 160 dB re. 1 μPa^2 s at 250 clicks s^{-1} (lq 159, uq 161); median: 156 dB re 1 μPa^2 s^{-1} at 50 clicks s^{-1} (lq 156, uq 158); and median: 156 dB re. 1 μPa^2 s at 20 clicks s^{-1} (lq 154, uq 156)]. There was no significant difference in the median EFD at 20 *versus* 50 clicks s^{-1} (P=0.248, Wilcoxon test, N=10, n=3), but there was a significant difference in the received EFD between 250 and 50 clicks s^{-1} (P=0.015, Wilcoxon test, N=10) and between 250 and 20 clicks s^{-1} (P<0.01, Wilcoxon test, N=10).

Swimming speed

When exposed to ultrasound stimuli played at repetition rates of 20, 50 and 250 clicks s^{-1}, shad increased their swimming speed. There was no change in swimming speed, when exposed to a single click. At the highest repetition rate (250 clicks s^{-1}) the median speed approximately doubled after exposure compared with pre-exposure (Table 1). C-bends were observed in all cases of significant increase in swimming speed. The difference in swimming speed pre and post sound exposures was significant at 250 clicks s^{-1} (Wilcoxon test, P<0.01, N=10, n=4), 50 clicks s^{-1} (Wilcoxon test, P<0.01, N=10), 20 clicks s^{-1} (Wilcoxon test, P<0.01, N=10), but not at 1 click s^{-1} (P=0.88, Wilcoxon test, N=10). There was a significant difference in median swimming speed after exposure at 250 *versus* 20 clicks (P<0.01, Wilcoxon test, N=10, number of tests=3) but not at 50 *versus* 20 clicks s^{-1} (Wilcoxon test, P<0.04, N=10), nor at 250 *versus* 50 clicks s^{-1} (P=0.2, Wilcoxon test, N=10).

Response direction

We investigated whether the fish exhibited negative phonotaxis with respect to the directional underwater transducer by measuring the angle between the axis of the sound source and the swimming direction of the fish in steps of 100 ms, starting at the first sign of

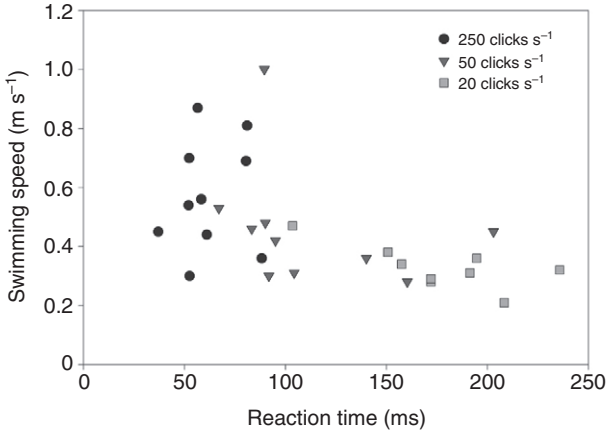

Fig. 3. The reaction time of allis shad plotted against swimming speed. Black, red and green symbols are responses of fish exposed to 250, 50 and 20 clicks s^{-1}, respectively, at a constant sound pressure level (SPL) of 192 dB re. 1 μPa (pp).

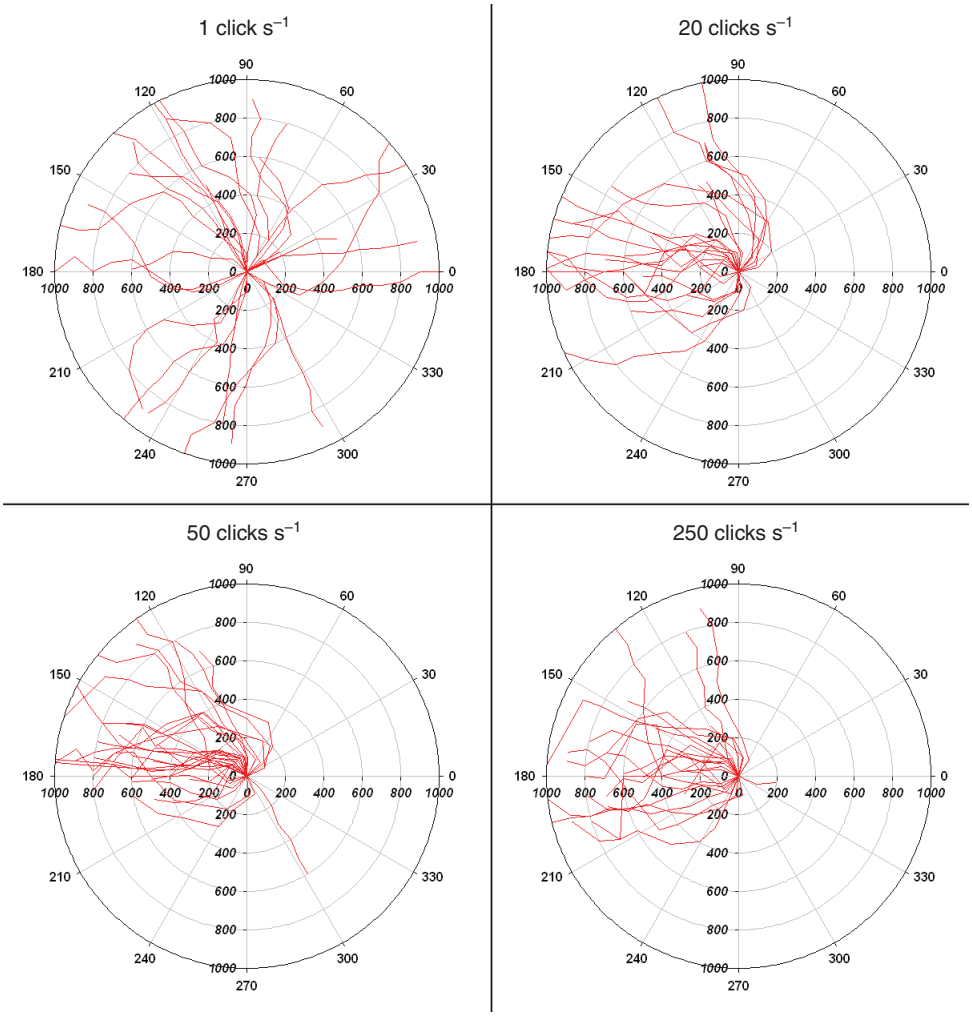

Fig. 4. Directional response of allis shad exposed to ultrasound. The polar plots show the angle between the sound source and the swimming direction of the fish exposed to four different repetition rates of ultrasonic clicks. 0 deg indicates the direction towards the sound source. *X*- and *Y*-axes are time in ms. The fish was exposed to ultrasound at time 0 ms. All four plots are based on data from 10 fish exposed to the different repetition rates with a received sound pressure level of 192 dB re. 1 μPa (pp). Each fish was exposed to the sound source two or three times with different initial swimming directions (1 click s^{-1}: *N*=27; 20 clicks s^{-1}: *N*=24; 50 clicks s^{-1}: *N*=25; 250 clicks s^{-1}: *N*=23). The traces are not tracks of the fish, but the swimming direction as a function of time in 50 ms bins after stimulus onset.

response to the ultrasound and continuing until the fish disappeared from the view of the camera. If there was no sign of reaction (when the fish was exposed to a single click) measurements of swim directions started at the frame of sound emission. A more detailed analysis of ten video sequences was performed (one from each fish when exposed to the 250 clicks s^{-1} exposure) where the angle was measured every 10 ms, showing that the 100 ms analysis steps provided adequate resolution.

At EFD levels above response threshold, allis shad showed a directional response (Figs 4 and 5). There was a tendency for the fish to escape by swimming away from the sound source, i.e. at an angle of approximately 180 deg independent of the initial angle to the transducer (Figs 4 and 5). At the three highest repetition rates 71, 74 and 74% of the fish escaped at angles of 180±30 deg relative to the transducer, whereas at the lowest repetition rate (1 click s^{-1}) none of the fish showed a directional response, and only 15% swam in the direction of 180±30 deg relative to the transducer (Fig. 4), as would be expected if the direction of swimming was random. A test for binomial distribution was significant at 20, 50 and 250 clicks s^{-1} (all at $P<0.01$, N=24, 25 and 23, respectively), but not at a 1 click s^{-1} (P=0.44, N=27). At 20, 50 and 250 clicks s^{-1} there seemed to be a bias for the fish to escape the sound source at an angle of 135 deg to 180 deg compared with 180 deg to 225 deg. At 20 and 50 clicks s^{-1} there was a significant difference between the two binomial distributions ($P<0.05$ and 0.05, respectively), but not at 1 or 250 clicks s^{-1} (P=0.23 and 0.33, respectively). In Fig. 4 it is seen that not all of the traces continued to the edge (especially for the fish exposed to 250 clicks s^{-1}). When the trace stops, the fish was outside the view of the camera. At 250 clicks s^{-1} the fish had a higher swimming escape speed (Table 1). This explains why more of the traces do not go to the edge in this case.

Control experiments

All three allis shad used for the control responded strongly when exposed to the unfiltered ultrasonic signal but they did not respond to the low-pass filtered version of the signal. Also, when triggering the recording system without connecting the playback system, none of the allis shad responded.

None of the goldfish showed any behavioural response when exposed to the ultrasonic clicks from the transducer. All five tested goldfish showed a startle response when exposed to a low-frequency sound stimulus in the form of a knock on the tank, demonstrating intact hearing.

DISCUSSION

This study demonstrates that allis shad exhibit highly directional behavioural responses when exposed to trains of ultrasonic clicks at 40 kHz with repetition rates mimicking toothed whales during the different stages of pursuit: search, approach and capture of prey.

Two control experiments verified that it was indeed the ultrasonic component of the sound field that allis shad responded to. Allis shad

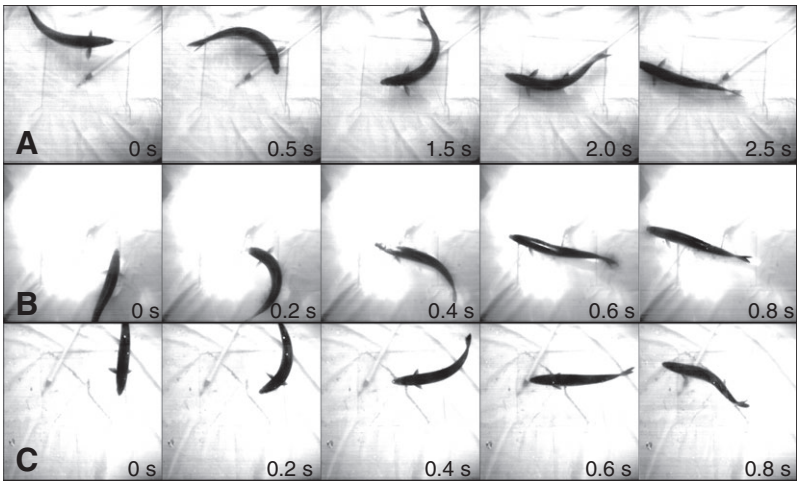

Fig. 5. Examples of the directional response of allis shad to ultrasound exposure. Response patterns of three fish approaching the transducer from three different angles. The directional sound source was situated to the right. Time is shown in the bottom right. The fish was exposed to ultrasonic sound at time 0.0 s. (A) Exposure to clicks played at a repetition rate of 20 clicks s^{-1} while the fish is swimming directly towards the transducer. (B) Exposure to clicks played at a repetition rate of 250 clicks s^{-1}. The fish is approaching the transducer with its right side toward the transducer. (C) Exposure to clicks played at a repetition rate of 250 clicks s^{-1}. The fish is approaching the transducer with the left side toward the transducer.

did not exhibit any reactions when exposed to a low-pass filtered version of a click train, nor did the test of a goldfish in the setup elicit any reactions.

The ability to tell the direction of an incoming predator is essential for survival (Karlsen, 1992). When allis shad are exposed to ultrasound, their response is consistent with a predator avoidance response, i.e. they turn away from the sound source (Fig. 4). Earlier studies have shown that shad turn away when ultrasound is emitted from an omnidirectional transducer (Plachta and Popper, 2003; Wilson et al., 2008); however, the directional response patterns of those studies were not analysed in details. In the present study a directional transducer was used, and we found that the fish swam away from the sound source at an angle of 180±30 deg, independent of the orientation of the fish relative to the transducer at the start of the sound exposure (Figs 4 and 5). When moths are exposed to low-intensity ultrasonic bat cries, they always turn directly away from the sound source, hence increasing the distance from the bat (Roeder, 1962). If allis shad exhibit the same behaviour in the wild as in these playback experiments (given the potential caveats associated with a tank *versus* the wild) by turning away from the incoming sound source, it will not only move the fish further away from the predator, but also change its detectability for the toothed whale biosonar. By swimming away at an angle of 180 deg, the fish is ensonified from the tail aspect, which will decrease the target strength of the fish by up to 14 dB compared with a broadside aspect (Au et al., 2007). This will reduce the shad detection range for the toothed whale by more than a factor of two compared with if the shad was ensonified from a side aspect, given that the echo intensity falls off by 12 dB per doubling of distance. Thus, the response of turning away from the sound source not only increases the distance between the fish and its predator, but also renders the fish a much harder target to detect, and may as such be an example of active acoustic crypsis (Madsen et al., 2007).

When the fish are swimming away from the sound source, there is a bias for the fish to swim in the direction of 135 deg to 180 deg compared with 180 deg to 225 deg (Fig. 4). The bias toward the direction of 135 deg to 180 deg is significant at 20 and 50 clicks s^{-1}, but not at 250 clicks s^{-1}. A possible explanation is that the fish are trying to swim against the water current (imitating their natural behaviour of swimming up against the river current), as swimming in the direction of 180 deg – 45 deg is against the water current in the test tank. When the fish are exposed to 250 clicks s^{-1} it is comparable to the biosonar output of a toothed whale just before prey capture (a few metres from the fish). The trade-off between evading danger and going against the current may depend on an evaluation of the risk of danger, and at 250 clicks s^{-1} the escape behaviour seem to prevail (Skals et al., 2005). There is a tendency for the fish to have a slower turning rate when they are exposed to 20 clicks s^{-1} compared with 250 clicks s^{-1} (Fig. 4). It might be difficult to tell the distance to a sound source based on a single or few short clicks. When the fish are exposed to 20 clicks s^{-1}, they will have received 2 clicks after 100 ms, which may not be enough to identify the direction to the sound source. However, at 250 clicks s^{-1} the fish will have received 25 clicks after 100 ms, which probably allows for a faster precise localization, explaining why they turn away from the sound source at a rate that increases with repetition rate.

There was no behavioural response of allis shad to single clicks, whereas fish exposed to the same sound pressure level at repetition rates of 20, 50 and 250 clicks s^{-1} showed clear reactions. These observations can be interpreted in several ways. One explanation is that the fish process the information given by the frequency of the click exposures, and in that way they can discriminate between different click repetition rates impinging on it (Astrup and Møhl, 1997). When the fish do not respond to a single ultrasonic click, it is not necessarily because they do not detect it, instead they do not interpret a single click as danger. They might detect it, but simply ignore it, as is seen in the moth and bat interaction (Roeder, 1962), where short single pulses above sensory threshold always lead to physiological responses of the sensory receptors of the ear, but never to behavioural responses (Skals and Surlykke, 2000) Alternatively, this response pattern may not necessarily demonstrate discrimination, but rather be a consequence of how the sensory system process incoming stimuli. A simple explanation for the lack of responses to a single click, but strong responses to multiple clicks of the same sound pressure, could be that the ultrasonic sensory system responds to energy rather than pressure. All vertebrate hearing organs, including the cod ear (Hawkins, 1981) and moth ear (Tougaard, 1996), perform temporal integration of sound intensity (Green, 1985), so it is not surprising if the same seems to hold true for the ultrasonic detector of allis shad. If the ultrasonic detector in shad operates as an energy detector, the fish should respond when a certain energy level is reached. As seen from Table 1, the EFD levels at the time of response are around 157 dB re. 1 μPa^2 s for the ten fish. However, it apparently takes 4 dB more energy for the fish to respond at 250 clicks s^{-1} compared with 20 clicks s^{-1}. This difference is small and may relate to scatter in the measurements. If genuine, the explanation for this difference may be that the time

from the onset of exposure to the time of behavioural response is the sum of the integration time of the detector as well as the time it takes for the fish to execute its motor pattern after the sensory threshold is reached and thereby meet our behavioural threshold for a response. Assuming that there is a constant delay, from the time when the energy threshold is reached, to the time of the behavioural response of the fish, the differences in cumulated energy at the three repetition rates will be zero if the combined time to reach our response threshold is around 40 ms, i.e. somewhat longer than the minimum delay of the C-start (Eaton et al., 1977). That would yield a minimum integration time of approximately 142 ms (182–40 ms) in the ultrasonic detector, which is in general agreement with the hearing integration times found across several taxa of around 200 ms (Ehret, 1976; Hawkins, 1981; Plomp and Bouman, 1959; Surlykke et al., 1988).

If the ultrasonic detector is an energy detector it is not reasonable to compare pressure thresholds for clicks (Mann et al., 1998) and pressure threshold for long pure tones without taking the duration of the signal into account (Mann et al., 2001; Mann et al., 1998; Mann et al., 1997; Plachta and Popper, 2003; Wilson et al., 2008). The EFD thresholds should rather be compared, keeping in mind the integration time of the ultrasonic detector. To further test the hypothesis that the ultrasonic detector is an energy detector, future click exposure studies should include sound exposure at different amplitudes.

In acoustic interactions between allis shad and their predators, a single click or a series of weak clicks at a low repetition rate does not necessarily pose danger, since the clicks are most probably emitted by a scanning whale far away. Real danger arises when the toothed whale has locked its biosonar onto the fish and approaches for capture. If the ultrasound detector is an energy detector, the threshold can be reached by slow clicking with high sound pressure or fast clicking at lower sound pressure. That feature will accommodate the fact that toothed whales click slowly and with high source levels at long ranges from their prey, but switch to a very fast repetition rate buzzing of lower sound pressure just before prey capture when they are within a body length of the prey (Deruiter et al., 2009; Madsen et al., 2005). With the estimated response energy thresholds one can use the passive sonar equation (Urick, 1983) to estimate at what range an allis shad will be able to detect a toothed whale, for instance a bottlenose dolphin. A bottlenose dolphin in an approach phase (about 10 m away) may emit clicks at a repetition rate of 50 clicks s^{-1} with source energy flux densities of 165 dB re. 1 μPa^2 s per click (Au et al., 2007). If we further assume that the integration time of the ultrasound detector in allis shad is 200 ms, then the dolphin will create an on-axis energy exposure of 175 dB re. 1 μPa^2 s 200 ms^{-1} (Au et al., 2007). Given the estimated response thresholds for allis shad of 145–157 dB re. 1 μPa^2 s, this would result in predator detection ranges of between 10 and 50 m if the dolphin sonar is locked on the fish. Based on these estimates it would be possible for allis shad to detect the toothed whale somewhere during the approach phase and before the toothed whale enters the final prey capture phase, where it is only some few meters from the fish.

Behavioural studies performed by Plachta and Popper (Plachta and Popper, 2003) and Wilson et al. (Wilson et al., 2008) showed that American and allis shad exhibit a graded response depending upon the sound level. We also find this graded pattern in the swimming speeds (Fig. 3 and Table 1), with the highest swimming speeds elicited by the highest repetition rates. However, the swimming speeds found were in general lower than in the two previous studies, which we ascribe to the fact that the fish were exposed to varying click trains, whereas Plachta and Popper (Plachta and Popper, 2003) and Wilson et al. (Wilson et al., 2008) used long pure tone signals with a higher overall energy content.

The EFD thresholds for a response are relatively high, but detection may happen at lower energy levels. Previous studies of the interaction between fish and their predators have shown that when facing a predator, the fish does not perform a fast-start escape response when just detecting the predator; only when the predation risk increases through a close up interaction will the fish exhibit the maximal escape response (Magurran and Pitcher, 1987). Compared with sensory thresholds, the behavioural response thresholds are also relatively high in moths (Surlykke et al., 2003), as is the case with allis shad, but sensitive enough to allow time to make an escape response (Miller and Surlykke, 2001).

CONCLUSION

The present study shows that the ultrasound detection system in Alosinae results in directional evasive manoeuvres, which presumably reduce the risk of predation from echolocating toothed whales. In a test tank, allis shad swam away from a sound source projecting ultrasonic pulses. Thereby the shad increased the distance to the sound source and, perhaps just as important, decreased the backscatter by changing their own exposure angle by turning their tail toward the sound source, thereby halving the potential detection range for the toothed whale. Allis shad show different reaction times when exposed to different repetition rates of echolocating signals of the same sound pressure. The faster the repetition rates, the faster the reaction time and swimming speed. A possible explanation for this observation may be that the ultrasonic detector is an energy detector, integrating sound intensity. A behavioural threshold requiring a high EFD level will ensure that shad only execute escape responses to nearby toothed whales that have locked their sonar on the fish. The results are consistent with studies on acoustic interactions between bats and their prey, showing that the interaction in air and water has converged evolutionarily not only in the way the predators operate their biosonar but also in the way some specialized prey species seem to have evolved ultrasound detectors in response to the acoustic signature of a potential predator.

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