

The responses of Atlantic cod (*Gadus morhua* L.) to ultrasound-emitting predators: stress, behavioural changes or debilitation?

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SUMMARY

A previous study has reported that Atlantic cod can be conditioned to detect ultrasonic sound pulses of high intensity. This capability has been proposed as a mean for detection and avoidance of echolocating toothed whales that emit intense ultrasonic clicks. In this study, we use acoustic playbacks to test the hypotheses that unconditioned cod can detect and respond to intense ultrasound from toothed whales and from echosounders. Intense ultrasound exposure of 210 dB re. 1 μ Pa (pp) did not cause a short-term stress response in the form of bradycardia in unconditioned cod. Free-swimming cod exposed to ultrasonic clicks and echosounder pulses with received levels of more than 204 dB re. 1 μ Pa (pp) did not elicit flight responses as seen in ultrasound detecting Alosinae. Furthermore, we tested the debilitating effects of high intensity ultrasound on swimming cod with no detected changes in swimming ability when exposed to more than 213 dB re. 1 μ Pa (pp). It is concluded that intense ultrasound exposure induces neither an antipredator nor a stress response in Atlantic cod, and that echosounder pulses and biosonar clicks therefore most probably play no ecophysiological role in wild cod populations.

INTRODUCTION

Predator–prey interactions are dependent on the sensory organs, the motor capabilities and the behaviour of prey and predators. Predators must probe their environment to find and catch prey, while potential prey items must search for food and reproduce while maintaining the capability to detect and avoid their predators. Prey will therefore be affected by predators in ways other than direct predation in the forms of stress (Remage-Healey et al., 2006), and changes in behaviour and in life history strategies (Lima and Dill, 1990). Predators can thus potentially cause stress and behavioural changes in a much greater number of prey than they successfully track and catch (Ripple and Beschta, 2004).

The use of intense ultrasound to echolocate fish and squids has been employed by toothed whales since the Oligocene between 37 and 28 million years ago (Fordyce and Barnes, 1994). Therefore, a significant evolutionary pressure (Clarke, 1996) has been present that may have induced the development of ultrasound detection in fish that, like the Atlantic cod (*Gadus morhua*), are preyed upon by toothed whales (Santos et al., 2001). Such ultrasonic interfaces in the sensory arms race between predators and prey have been documented for bats and moths (Miller and Surlykke, 2001), and also suggested for toothed whales and their prey (Astrup, 1999; Wilson et al., 2007).

Recently, on an evolutionary timescale, humans have been added to the list of ultrasound-emitting predators that target cod. The Atlantic cod has been a very important fish in world fisheries for more than five centuries (Jónsson, 1994; Øiestad, 1994), and are now commonly sought using echosounders with source level sound pressures up to 230 dB re. 1 μ Pa (pp) (Simmonds and MacLennan, 2005) – comparable in peak pressure with the maximum levels produced by toothed whales (Au, 1993). If Atlantic cod has developed ultrasonic hearing in response to predation from echolocating toothed whales (Astrup, 1999), any ultrasound detected

above a specific threshold should elicit an anti-predator response and therefore also be a source of potential short- or long-term stress. Artificial ultrasonic sound sources, such as echosounders would then be expected to have similar negative effects on cod, possibly along with ultrasound pingers used to reduce the by-catch of small cetaceans (Kastelein et al., 2007).

Most cod stocks in the north Atlantic have undergone severe decreases in numbers (Garrod and Schumacher, 1994) and strict management has been implemented in an attempt to sustain population numbers. Although this management has mostly focused on reducing catches through minimizing quotas, stress and behavioural changes have received little attention. These issues should be taken into consideration as they have implications for both recruitment (Morgan et al., 1999) and vulnerability to diseases (Pickering and Pottinger, 1989; Schreck, 1996).

In fact, Astrup and Møhl (Astrup and Møhl, 1993) did report that Atlantic cod can be conditioned to detect high intensities of ultrasound and suggested that this capability evolved to detect and avoid echolocating toothed whales (Astrup, 1999). Through conditioning with mild electrical shocks, they determined the detection threshold to be 203 dB re. 1 μ Pa (pp) for an echosounder pulse at 38 kHz, and on those grounds estimated that a cod should be able to detect an approaching toothed whale at a range of 10–30 m (Astrup and Møhl, 1993). Atlantic cod in the North Sea are commonly found at depths less than 30 m and echosounders should thus be detectable by cod throughout the entire water column when ensonified by the beam of the downward-directed transducer. From the findings of Astrup and Møhl (Astrup and Møhl, 1993), it follows that ultrasound-detecting cod should react to ultrasound-emitting predators if this capability has evolved as an anti-predator response. Such anti-predator responses have recently been documented for another family of fish: the ultrasound-hearing shad (Alosinae) species (Mann et al., 2001;

Mann et al., 1998; Wilson et al., 2008). In addition, much stronger effects of ultrasound have been proposed by Norris and Møhl (Norris and Møhl, 1983) who advanced the hypothesis that very high intensities of ultrasound might have a momentary debilitating effect on fish that could facilitate capture.

In this study, we test these hypotheses on the effects of high-intensity ultrasound on cod and discuss the findings in the light of toothed whale echolocation and fisheries acoustics. We test the effects of intense ultrasound in terms of a short-term stress response, behavioural responses and acoustic debilitation. It is demonstrated that intense ultrasound does not elicit short-term stress or anti-predator responses, and that very high sound intensities have no apparent debilitating effect on Atlantic cod. Contrary to previous studies, it is concluded that intense ultrasound most probably plays no role in predator-prey interaction between cod and their ultrasound-emitting predators.

MATERIALS AND METHODS

Animal collection and maintenance

Three experiments were carried out to test the effects of high intensity ultrasound on Atlantic cod (*Gadus morhua* L.).

(1) We tested whether detection of high-intensity ultrasound could induce a short-term stress response, as reflected in the heart rate. If the fish detect and perceive ultrasound as a potential threat, we expected to see bradycardia that is a characteristic of the orienting response (Knudsen, 1994).

(2) We investigated the effect of high-intensity ultrasound on the behaviour of free-swimming cod and looked for anti-predator responses, such as strong avoidance.

(3) We tested whether very high intensities of ultrasound could have a debilitating effect on cod during sustained swimming in a flow chamber.

The experiments took place in the period October 2006 to July 2007 on Atlantic cod caught in the North Sea off Denmark. Heart rate and debilitation experiments were carried out in a 3×4×6.5 m sea water tank (Figs 1 and 2) at the North Sea Museum in Hirtshals. Behavioural experiments were conducted at the Fisheries and Maritime Museum in Esbjerg in a 3.35×3.5×10 m exhibition aquarium. All cod were allowed to recover in holding tanks at least 1 month prior to experimentation, and all displayed normal behaviour and were feeding prior to the experiment.

Heart rate experiment

Ten Atlantic cod with an average length of 36±4 cm were used in this experiment.

The fish were anaesthetized in a 0.4 mmol l⁻¹ benzocaine (Sigma) solution and placed ventral side upwards on an operating desk. Teflon-coated stainless steel electrodes (125 µm in diameter) were placed on either side of the heart by inserting a syringe holding the tip of the electrode under the pelvic girdle and pushing it anteriorly approx. 2 cm. When the electrode was in place, a loop was made on the electrode just where it penetrates the skin. A suture was made through the loop on both electrodes, thus keeping them in place and, regardless of behaviour of the fish, preventing it from pulling the electrodes out during the experiment.

The fish was woken from anaesthesia through artificial gill ventilation by moving the fish back and forth in the water. As soon as the fish was able to ventilate the gills on its own in a stable manner, it was placed in the test restrainer, where it was left to recover for 15–24 h (Axelsson, 1988). Heart rate data were collected using an AcqKnowledge MP 100 data-acquisition system sampling at 200 Hz (BioPac systems, Santa Barbara, CA, USA). The ECG signal was

amplified using a custom-built pre-amplifier. After the recovery period, the fish were exposed to one of two experimental treatments. Five fish were exposed to a set-up in which the test restrainer was placed 1.05 m in front of a Simrad EK-38/22E echosounder transducer. Using an Agilent 33220A arbitrary waveform generator and a custom-built amplifier, 50 kHz sound pulses with a duration of 10 ms and a repetition rate of 10 pulses s⁻¹ were generated,

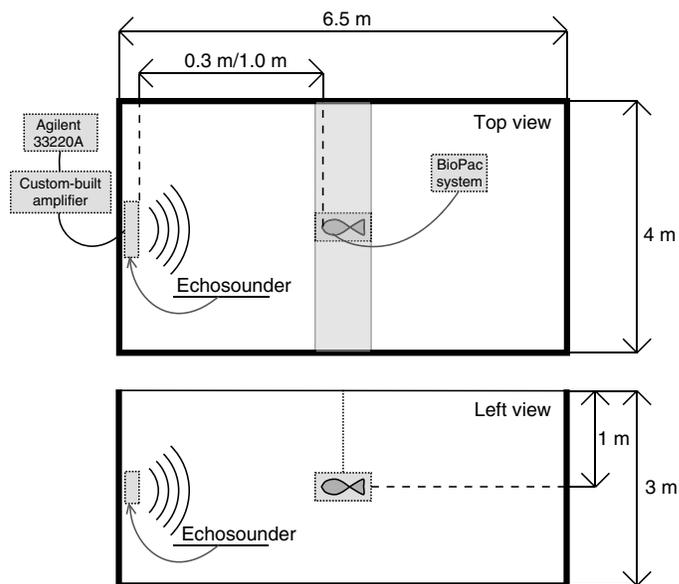


Fig. 1. The experimental set-up of the heart rate experiment. Proportions are not accurate. The thick black lines indicate the sides of the test tank. The electrodes are connected to a custom-built pre-amplifier connected to the BioPac system. The restrainer containing the fish is placed 1.05 m in front of the transducer during the far-field exposures and 0.3 m in front in the near-field set-up.

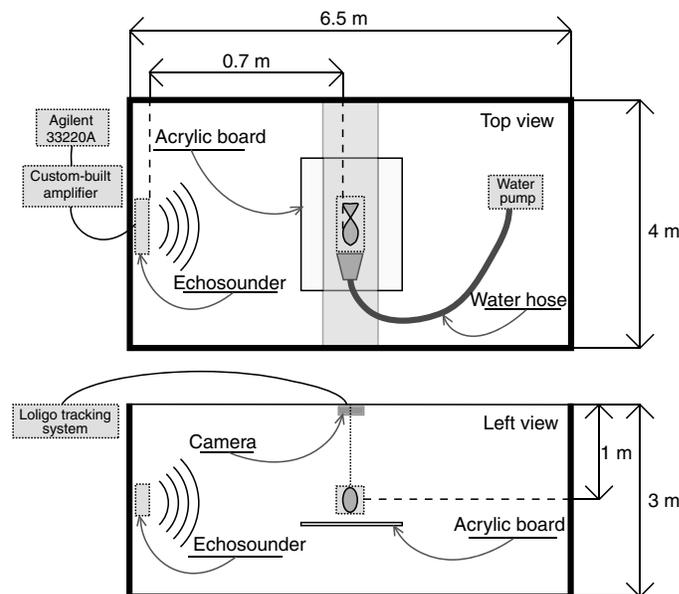


Fig. 2. The experimental set-up for the debilitation experiment. Proportions are not accurate. The thick black lines indicate the sides of the test tank. The flow chamber is placed parallel to the impinging sound wave and is positioned so the head of the fish is in the middle of the acoustic beam.

mimicking a pulse type commonly used by echosounders (Simmonds and MacLennan, 2005). The received sound pressure level at the fish in the restrainer was measured to be 213 ± 1 dB re. $1 \mu\text{Pa}$ (pp) using a calibrated Reson TC 4034 hydrophone (Fig. 3). The exposure period was 30 s, yielding a sound exposure level (SEL) of 184 dB re. $1 \mu\text{Pa}^2\text{s}$ (1.6 J m^{-2})[†] for a single pulse and 209 dB re. $1 \mu\text{Pa}^2\text{s}$ for the entire 30 s exposure period. To ensure that the fish were fit and able to display a reaction that would be detectable in their heart rate, they were either exposed to one of two different stimuli: one of the experimenters becoming visible to the test animal or exposure to a low-frequency acoustic stimulus (a knock on the mount holding the restrainer). The experimental session ended by removing the fish from the restrainer and euthanizing it with a blow to the head.

To have control of the sound field in terms of both pressure and particle acceleration, the sound exposure experiments were carried out in the acoustic (Frauenhofer) and in the flow far-fields of the sound source. In this experiment, another five fish were exposed using a second set-up, where the test restrainer was only 30 cm from the transducer, which mimics the set-up of Astrup and Møhl (Astrup and Møhl, 1993). This range to the transducer placed the fish in the pressure (Fresnel) near-field of the transducer, but outside the flow near-field. The flow near-field is defined as the distance from the transducer at which the particle motion cannot be predicted from the pressure component of the sound field (Urlick, 1983). If the product of kr , where k is the wave number and r is the distance between the sound source and the receiver, is much greater than 1, the restrainer could be assumed to be outside the flow near-field. The kr product for a range of 30 cm was calculated to be 63, at which the restrainer is considered to be well outside the flow near-field of the transducer. The second type of near-field is the Fresnel pressure near-field. This is the distance from a composite sound source within which the sound source can no longer be regarded as being an acoustic point source. The distance is calculated as:

$$r = (2a)^2 / \lambda,$$

where a is the radius of a piston assumed to have the same radiation pattern as the sound generator, and λ is the wavelength (Urlick, 1983). At distances shorter than this, cancellations can occur, making nodes and notches, and yielding an uneven sound-pressure field. The Fresnel near-field was in this instance calculated to be 85 cm, so a distance of 30 cm puts the fish well within the Fresnel near-field, which is likely to generate significant pressure differences along the fish body for pure-tone pulses.

For the 30 cm set-up, the received sound-pressure level at the head of the fish was measured to be 216 ± 1 dB re. $1 \mu\text{Pa}$ (pp) using a B&K 8103 hydrophone. The exposure period was again 30 s, giving an SEL of 187 dB re. $1 \mu\text{Pa}^2\text{s}$ for a single pulse and 212 dB re. $1 \mu\text{Pa}^2\text{s}$ for the entire exposure. Fifteen minutes after experimental exposure, the fish was exposed to the control, consisting of either a visual stimulus or a knock on the mount holding the restrainer. The fish was then euthanized with a blow to the head. Acoustic and heart rate data were processed using Matlab v. 6.5 (Mathworks, Inc., Natick, MA, USA) and the statistical analysis was carried out with the statistical toolbox in Matlab v. 6.5.

[†]For a plane wave in an unbounded medium, the energy flux density in dB re. $1 \mu\text{Pa}^2\text{s}$ can be converted to J m^{-2} by dividing the summed squared pressure on a linear scale by the specific impedance Z (sound speed \times density) of the medium. To exemplify, $182 \text{ dB re. } 1 \mu\text{Pa}^2\text{s} = (1580 \text{ Pa}^2\text{s m}^{-2}) / (1500 \text{ m s}^{-1} \times 1040 \text{ kg m}^{-3}) = 1 \text{ J m}^{-2}$.

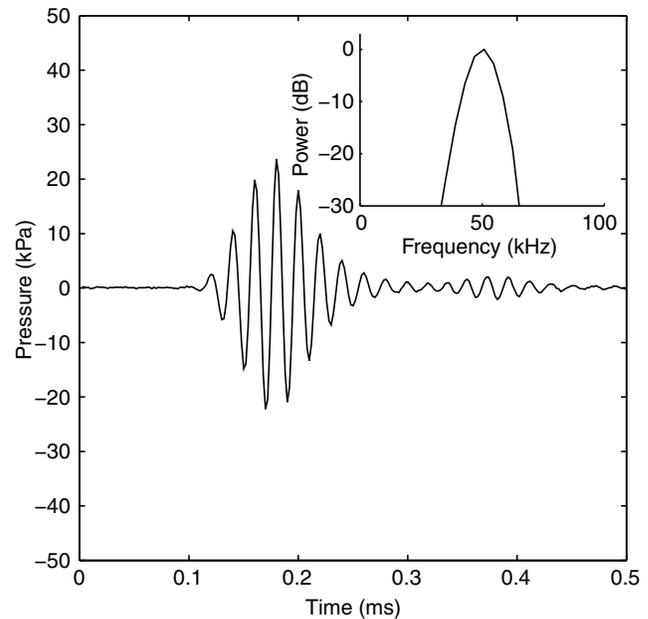


Fig. 3. Wave form and power spectrum (inset) (FFT size 128, sampling rate 500 kHz, hamming window) of the pulse used in heart rate, behavioural and debilitation experiments. The signal was recorded inside the test tank in Hirtshals at the position of the fish.

Behavioural experiment

The experiment was conducted in an aquarium containing six Atlantic cod. Exposures consisted of 50 kHz pulses with duration of 10 ms and a repetition rate of 10 pulses s^{-1} . The exposure period was 1 s, and consisted of 10 pulses. The stimulus was generated using an Agilent 22330A arbitrary waveform generator connected to a custom-built amplifier and a Reson 2116 broadband transducer.

The exposure zone was monitored using two Profile underwater cameras connected to laptops through Grabster AV400 A/D cards. One of the cameras was mounted on top of the transducer, thereby enabling the monitoring of fish passing in front of the transducer. A square measuring $1 \times 1 \text{ m}$ was placed at the far wall opposite the transducer. This was used to indicate the area viewed by the camera within which the fish was ensounded by the calibrated acoustic beam. The other camera was placed so as to give an aerial view of the area in front of the transducer where the fish would be exposed to between 208 dB re. $1 \mu\text{Pa}$ (pp) and 220 dB re. $1 \mu\text{Pa}$ (pp). The sound field was measured using a calibrated Reson TC 4034 hydrophone with a flat (± 2 dB) frequency response from 1 to 150 kHz.

The cod was stimulated when in view of both cameras. A change in swimming behaviour was quantified by comparing the maximum tail-beat amplitude before and after the onset of exposure. This was carried out by analysing the video recordings of the swimming fish frame by frame. For each tail beat, the frame where the tail of the fish was displaced maximally from the longitudinal axis of the body was saved as an isolated picture. On each of these frames, a line was drawn through the two points that were given by the most anterior point of the head and by the beginning of the dorsal fin. This line indicated the main axis of the body from which the degree of tail displacement was measured. The distance from the tail to the main axis was measured using a ruler, and the distance was normalized by dividing it by the distance measured from the head to the beginning of the dorsal fin. Three tail beats before and three

tail beats after the onset of the exposure were analysed in this way using Ulead VideoStudio 7 and Pinnacle Studio Version 9 video editing software. Statistical analysis was carried out using the statistical toolbox in Matlab v. 6.5.

Debilitation experiment

For this experiment, ten Atlantic cod with an average length of 38 ± 4 cm (mean \pm s.e.m.) were used.

An acoustically transparent flow chamber was built using a Plexiglas cylinder with an inner diameter of 20 cm. The cylinder was capped at both ends with honeycombs to ensure an approximately even velocity profile across the diameter of the flow chamber. One end of the flow chamber was mounted with a conical section 30 cm in length that was connected to a submersible pump with a capacity of $26 \text{ m}^3 \text{ h}^{-1}$. The output of the water pump was set to create a water flow speed of approx. 0.4 m s^{-1} [measured with a flow probe (Höntzsch Instruments, Germany) in side the chamber] corresponding roughly to one body length. Each fish was introduced to the flow chamber 2 hours prior to the exposures. The water flow was started 30 min prior to sound exposure, after which the fish was assumed to have reached steady state.

Each fish was exposed to two treatments. Treatment one consisted of five cycle 50 kHz clicks with a duration of $100 \mu\text{s}$, a repetition rate of $200 \text{ clicks s}^{-1}$ to mimic the buzz phase of an approaching toothed whale (Madsen et al., 2005). A mean received sound pressure of $212 \pm 3 \text{ dB re. } 1 \mu\text{Pa}$ (pp) was measured inside the test tube in the volume occupied by the fish (Fig. 4). This yields an SEL of $161 \text{ dB re. } 1 \mu\text{Pa}^2 \text{ s click}^{-1}$ (0.01 J m^{-2}). The second treatment consisted of a 50 kHz pulse with duration of 10 ms, a repetition rate of 10 pulses s^{-1} with a mean sound pressure level of $214 \pm 3 \text{ dB re. } 1 \mu\text{Pa}$ (pp) yielding an SEL of $185 \text{ dB re. } 1 \mu\text{Pa}^2 \text{ s pulse}^{-1}$ (2.0 J m^{-2}).

Both treatments of 5 s duration were generated using an Agilent 33220A arbitrary waveform generator connected to a custom built amplifier and a Simrad EK-38/22E echosounder. These yielded

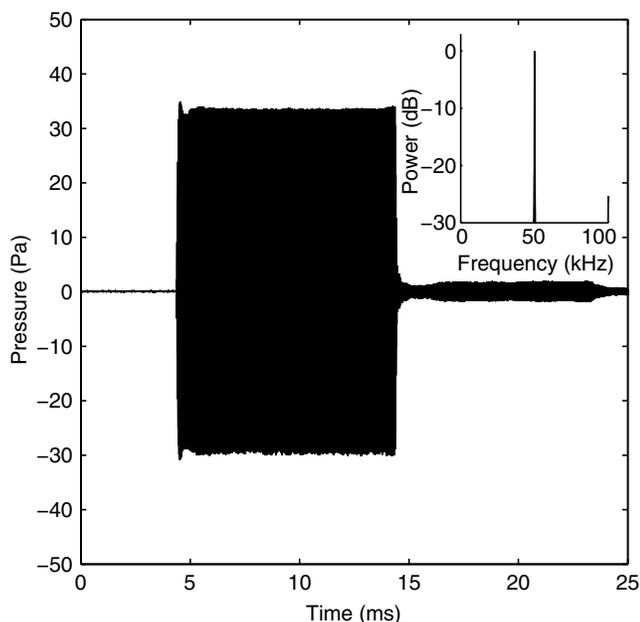


Fig. 4. Wave form and power spectrum (inset) (FFT size 8192, sampling rate 500 kHz, hamming window) of the click used in the debilitation experiment. The signal was recorded inside the test tank in Hirtshals at the position of the fish.

overall sound exposures of $191 \text{ dB re. } 1 \mu\text{Pa}^2 \text{ s}$ and $202 \text{ dB re. } 1 \mu\text{Pa}^2 \text{ s}$ for the click and pulse exposures.

After a swimming period of 30 min in the flow chamber, the fish was exposed to both treatments in random order 11 min apart to allow the fish a chance to recuperate after the first treatment.

The fish was tracked from the time swimming commenced and until 2 min after the last exposure using a vertically mounted LoliTrack 2D video tracking system (Loligo Systems ApS, Tjete, Denmark) sampling at 15 frames s^{-1} . To ensure good contrast between the fish and its background, a white acrylic board was placed underneath the flow chamber. The set-up was calibrated at the end of each session by placing a cross with known dimensions at the position of the fish. The tracking data consisting of x and y positions in the horizontal plane of the flow chamber was processed using Mathematica 5.2 (Wolfram Research, Champaign, IL, USA) and the statistical analysis was carried out in Matlab v. 6.5.

RESULTS

Three potential effects of ultrasound exposure were tested: (1) detection and display of acute stress responses in the form of bradycardia; (2) anti-predator behaviour through a behavioural startle response; and (3) acoustic debilitation. If high intensity ultrasound is detected, it is hypothesized that the maximum heart rate interval (HRI) will be significantly different during exposure from before, owing to the orienting response (Knudsen, 1994). Anti-predator behaviour is expected to yield a significant difference in the maximum tail-beat amplitude before and after the stimulation, in that a strong avoidance response would cause an increase in tail-beat amplitude. In the case of debilitation, the cod is kept swimming at a constant speed and if debilitation occurs, it is hypothesized that it will no longer be able to keep its position in the strong water flow.

Heart rate experiment

Ten Atlantic cod were exposed to ultrasound in either a pressure near- or far-field set-up. For both the near- and far-field treatments, the maximum HRI from the 30 s pre-exposure and the 30 s exposure periods are compared for all five fish in each treatment. For both the near- and far-field exposures, there is no significant difference in max HRI between pre- and exposure periods (near field, $T=5$; far field, $P=0.6250$; $T=3$, $P=0.3125$, Wilcoxon's test for matched pairs). As the heart rate pre-exposure periods for the two different treatments show both normal distribution ($P=0.6172$, Bera-Jarque parametric hypothesis) and have an equal variance ($P>0.05$), we pooled the data from the two treatments (Sokal and Rohlf, 1998). After pooling of data there was no significant difference between pre- and exposure periods ($T=27$, $P=1$, Wilcoxon's test for matched pairs). Fig. 5 shows the HRI of all ten fish during a 70 s. window around the exposure period, showing that no changes in HRI occur during the exposure period. For the control treatments, in which the fish 6, 8 and 10 have been exposed to the acoustic stimulation and the remaining fish have been exposed to the visual stimulation (Fig. 6) the maximum HRI before stimulation is significantly different from the maximum HRI afterwards ($T=0$, $P=0.002$, Wilcoxon's test for matched pairs).

Behavioural experiment

The maximum tail-beat amplitude from five cod was measured and normalized to the distance between the most anterior point of the fish and the beginning of the dorsal fin. Maximum tail beat amplitude was measured from three tail beats before the exposure and three tail beats after. The data are shown in Fig. 7. There is no

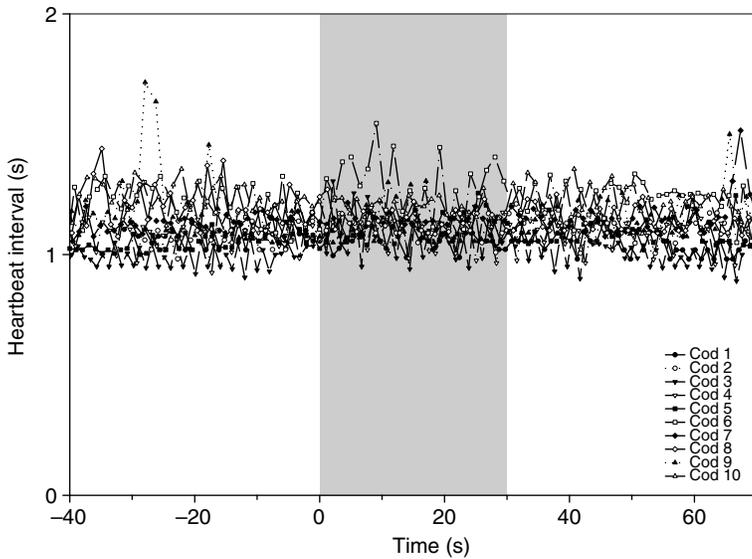


Fig. 5. The individual heartbeat intervals for 10 Atlantic cod exposed to ultrasound. The shaded area indicates the 30 s exposure period.

significant difference in maximum tail beat amplitude between the pre-exposure and exposure periods ($T=6, P=0.8125$, Wilcoxon's test for matched pairs). We observed no avoidance responses or C-starts in any of the cod exposed to ultrasound. We did, however, observe what appeared to be a mild response consisting of a stretching of the pectoral fins and a minor increase in swimming speed in a few fish at the onset of the exposure when they were very close to the transducer. There were, however, no consistent reactions and the reactions we did observe were not only seen in cod, but also in pollack (*Pollachius pollachius*), pouting (*Trisopterus luscus*), saithe (*Pollachius virens*) and sea bass (*Dicentrarchus labrax*) when they swam very close to the transducer in the same tank.

Debilitation experiment

Debilitation experiments were carried out on 10 Atlantic cod. Fig. 8A,B show the mean swimming speed of the 10 cod for click and pulse exposures, respectively. Data are presented in a 40 s window around the exposure. The fish was forced to swim at a constant speed of 0.4 ms^{-1} to keep position, and the deviation in

swimming speed from this value as seen in Fig. 8 is the second derivative of the positional data of the fish. If the fish was swept back in the flow chamber, it reflects an impaired swimming capability, thereby indicating debilitation. The maximum swim speed before exposure is not significantly different from the maximum swim speed during the experiment for either of the two treatments (click, $T=23, P=0.6953$; pulse, $T=18, P=0.3750$, Wilcoxon's test for matched pairs).

DISCUSSION

A number of clupeid fish species of the subfamily Alosinae detect and respond strongly to ultrasound, and playback studies have shown that ultrasound elicits strong anti-predator responses such as C-starts in the American shad (*Alosa sapidissima*) and the Allis shad (*Alosa alosa*) (Mann et al., 1998; Plachta and Popper, 2003; Wilson et al., 2008). Astrup and Møhl (Astrup and Møhl, 1993) also reported that Atlantic cod could be conditioned to detect ultrasound. In light of these studies, we wanted to test whether high intensity ultrasound evokes a response in unconditioned Atlantic cod in the form of

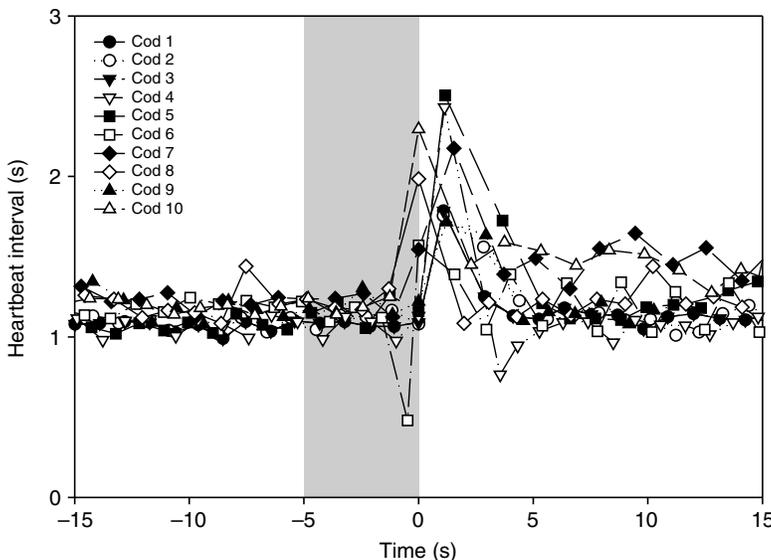


Fig. 6. Individual heartbeat intervals of 10 Atlantic cod during control stimulation. Fish 6, 8 and 10 were exposed to the acoustic control stimulation consisting of a hard knock on the mount holding the restrainer at $t=0$. The remaining fish were exposed to visual stimulation and the shaded area indicates the time frame within which the fish was stimulated visually.

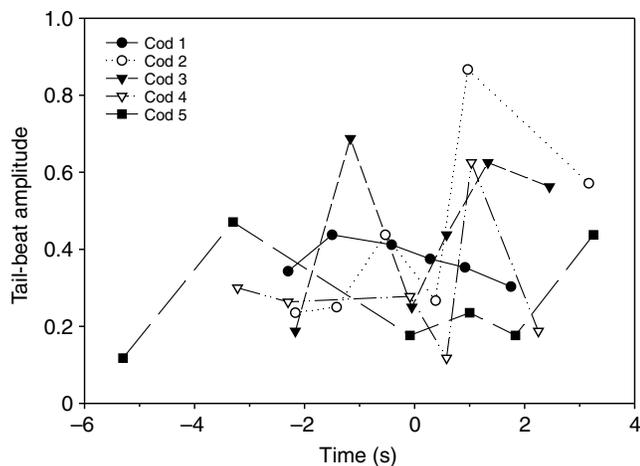


Fig. 7. The individual tail beat amplitude of five free-swimming Atlantic cod exposed to ultrasound. The shaded area indicates the 1 s exposure period.

bradycardia and avoidance responses, as would be expected if cod, like shad, were able to detect intense ultrasound as a part of an anti-predator measure.

In the heart rate experiment, we tested for any near-field effects of the sound exposure that could enable the fish to detect ultrasound. There are several mechanisms by which fish may detect such near-field effects. First, a fish can be regarded as a rigid body when placed parallel to the direction of the sound. Close to the sound source, at distances shorter than the length of the fish, the sound pressure will diminish significantly, so that the front and rear of the fish would experience different displacement. The head and tail should thus ideally move with different velocities, but as the fish moves as a rigid body it will move with an intermediate velocity, thus creating a difference in movement between the fish and the water at either end of the fish. This difference may be detected by the lateral line system (Sand, 1984). In addition, in the Fresnel near-field, because of the very high sound pressures used in this experiment, differences between nodes and internodes in the near-field could be very large and the mechanism mentioned above could thus be greatly enhanced. The tuning curve for the lateral line shows no sensitivity at frequencies as high as the ones used here, but, again, because of the very high sound intensities used, it is possible that the system might still be stimulated. We therefore tested the effects of ultrasound on the heart rate in both a pressure near- and a far-field set-up. There were, however, no changes in heart rate in either of the two set-ups.

Knudsen (Knudsen, 1994) showed that bradycardia is induced in Atlantic cod immediately after exposure to an acoustic stimulation consisting of a knock on the side of a holding tank, and heart rate is therefore a short-term measure of the fish being stressed or frightened by a given stimuli. We observed similar responses in our control experiments (Fig. 6). However, we never observed such a response in the heart rate data from the ultrasound exposure. If ultrasound was detected and perceived as a potential threat from an approaching predator, we hypothesize that bradycardia would have been the first indication, as it is a documented marker of the orienting response in fish, and thus the first reaction in a row leading to the flight or fight response (Sokolov, 1963). If bradycardia had been observed in our unconditioned fish, it would have implied that cod will probably be affected more or less every time intense ultrasound is detected

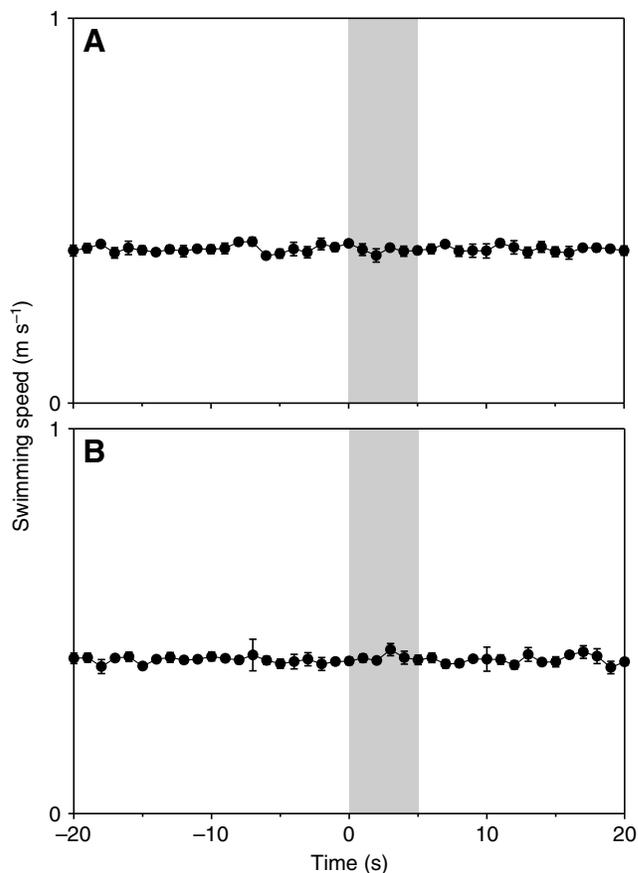


Fig. 8. Mean swimming speed of 10 Atlantic cod exposed to either an odontocete click (A) or an echosounder pulse (B). The shaded area indicates the 5 s exposure period.

from an odontocete or an echosounder. However, data from 10 cod clearly demonstrate (Fig. 5) that intense ultrasound at received levels of 213 ± 1 dB re. $1 \mu\text{Pa}$ (pp), which is well above the conditioned detection thresholds of 204 dB re. $1 \mu\text{Pa}$ (pp) reported by Astrup and Møhl (Astrup and Møhl, 1993), does not elicit any orienting response. The lack of orienting response shows that these cod do not associate ultrasound with predation attempts or other negative measures, despite the indication of normal hearing from the response to the low frequency knock. The fish were allowed to recover for a month after being caught. Complete regeneration of lost hair cells has been demonstrated to occur within a few weeks of them being damaged (Lombarte et al., 1993), so the hearing of the fish was therefore assumed to be normal.

To further test the assertion that cod do not respond to intense ultrasound, we conducted a series of playback experiments on free-swimming cod to test the hypothesis that cod would show an avoidance or C-start response if they, as inferred for shad (Plachta and Popper, 2003; Wilson et al., 2008), use ultrasound as a sensory cue to detect and evade ultrasound emitting odontocetes. If intense ultrasound elicits an avoidance response, significantly greater tail-beat amplitude would be expected after the onset of the stimulus. Analysis of video sequences shows that received levels between 208 and 220 dB re. $1 \mu\text{Pa}$ (pp) did not elicit avoidance responses in Atlantic cod (Fig. 7). However, in some cases the fish did seem to respond by spreading out their pectoral fins, and momentarily increasing their swimming speed slightly at the onset of the

stimulation, though it was not enough to significantly affect the tail-beat amplitude. This was, however, observed in only a few fish swimming very close to the transducer, and was thus not a consistent strong response, as is observed in shad at much lower received levels (between 140 and 180 dB re. 1 μ Pa) (Plachta and Popper, 2003). This inconsistency in reaction was also observed by Astrup (Astrup, 1997). The apparent response in the present study was seen not only in cod but also in pollack, pouting, saithe and sea bass in the same tank, and some members of all species showed the same type of response. In one instance, the mild response was elicited in two fish that were completely outside the ultrasonic beam but were right next to the transducer where the received levels from the ultrasonic pulse are more than an order of magnitude lower than in the beam. This suggests that the mild response seen in a few cases is not a response to the ultrasonic stimulation.

This then poses the question of what stimulus have these fish responded to? The Reson 2116 is a broadband transducer that also transmits low-frequency by-products, though at much lower outputs. The low-frequency components associated with the fast onset of a sound may therefore have been transmitted with a low directionality, possibly explaining why fish were observed to react outside the ultrasonic beam. Second, the peak-peak stimulus voltage of 140 V sent to the transducer would probably also create an electric field around it. In combination or separately, these two properties of stimulus could have caused the few observed responses rather than the ultrasonic exposure.

Given the lack of control in this behavioural experiment on the free-swimming fish, the possibility cannot be excluded that these five cod did not respond to ultrasonic exposure because of impaired hearing. However, in light of the consistent lack of responses in the heart rate experiment and in the behavioural experiment, we find it parsimonious to infer that the fish of the behavioural experiment did indeed have normal hearing, but did not respond to the ultrasonic exposure. In future experiments, control stimuli should consist of infrasonic sound pulses to which cod in opposition to mid-frequency stimuli (Kastelein et al., 2008) respond strongly (Knudsen, 1994).

Astrup and Møhl (Astrup and Møhl, 1993) reported a detection threshold for ultrasound in Atlantic cod of 204 dB re. 1 μ Pa (pp). The minimum exposure in the behavioural experiment conducted in this study is 3 dB above that threshold and in some cases more than 10 dB above it. This means that the intensity in the exposure is at least twice that of the threshold intensity found by Astrup and Møhl (Astrup and Møhl, 1993). Assuming that the cod in our study in fact had the detection threshold for ultrasound reported by Astrup and Møhl (Astrup and Møhl, 1993), we would thus expect a consistent anti-predator response if the ability of Atlantic cod to detect ultrasound indeed serves the same purpose as inferred for the ultrasound/predator detector found among the members of the Alosinae subfamily (Mann et al., 1998; Plachta and Popper, 2003). As neither bradycardia nor anti-predator responses are observed in Atlantic cod when exposed to ultrasound, the biological relevance of potential ultrasound detection is equivocal. We therefore, in contrast to Astrup and Møhl (Astrup and Møhl, 1993) and Astrup (Astrup, 1999), conclude that cod probably have not developed ultrasound detection capabilities to avoid predation from toothed whales, as suggested for shad (Plachta and Popper, 2003). The discrepancy between the findings from conditioned cod of Astrup and Møhl (Astrup and Møhl, 1993) and the present results from unconditioned cod remain unresolved, but it is possible that the cod used in the Astrup and Møhl (Astrup and Møhl, 1993) study, despite careful methodology, in fact were conditioned to artefacts rather than to the ultrasonic component of the exposure.

Ultrasound might, however, still play a role in the acoustic predator-prey interaction despite the lack of detection and behavioural responses in cod. The prey debilitation hypothesis advanced by Norris and Møhl (Norris and Møhl, 1983) proposes that very high intensities of ultrasound produced by toothed whales could have a debilitating effect on their prey. Toothed whales that capture prey increase their repetition rate, but lower their source level by about 10–20 dB when they are within a few meters of their prey (Madsen et al., 2005; Madsen et al., 2002), initiating what is termed the buzz phase (Miller et al., 1995). Source levels for most toothed whales do not exceed 230 dB re. 1 μ Pa (pp) (Au, 1993), so the maximum received levels at the prey would therefore not be expected to be higher than some 210 to 215 dB re. 1 μ Pa (pp) during any given point of ensonification from an approaching toothed whale predator.

Zagaeski (Zagaeski, 1987) showed that a broadband impulse of very high peak pressure of more than 230 dB re. 1 μ Pa (pp) generated by a spark generator had a debilitating effect on guppies. But although the sound pulse did contain ultrasonic components, it is very hard to discern whether ultrasound had any part in the debilitation, as the signal also contained very intense low-frequency components with very large particle motion component. Benoit-Bird et al. (Benoit-Bird et al., 2006) found no debilitating effects of ultrasound on Atlantic cod, Atlantic herring and sea bass. They used lower received levels than in the present experiment, and the parameters for testing debilitation could not quantify the escape and swimming capabilities of the fish, as the fish were restrained to a very small tank and did not have to swim to maintain position. Here, we tested whether swimming cod could maintain their position in a strong water current, thereby mimicking a situation in which a fish is chased by a predator. The experimental setup in the debilitation experiment forced the fish to swim at a constant speed. If debilitation occurred, we hypothesized that the ability of the fish to maintain its position and thereby its swimming speed would be impaired and that the fish would be flushed from its position by the water current. Such a change in position and thereby swimming speed would be clearly detectable in the tracking data, and thus provide an unequivocal test of whether the received levels had any debilitating effects on the escape capabilities of the cod. We found no debilitating effect of very high intensity ultrasound on Atlantic cod, as all 10 fish successfully maintained their position in the flow chamber at swimming speeds of more than 1 body length s^{-1} (Fig. 8). Thus, the present findings are not consistent with the debilitation hypothesis and it can be concluded that ultrasound exposures from odontocetes or echosounders do not debilitate Atlantic cod at received levels between 213 and 215 dB re. 1 μ Pa (pp).

Ultrasound is used for a variety of purposes in fishing and fisheries management, and if cod indeed were able to detect ultrasound, it could be a source of stress and behavioural disruption for areas heavily ensonified with ultrasound. Second, echosounders are used to estimate stock sizes (Michalsen et al., 1996), and behavioural effects of ultrasound exposure would therefore introduce a bias in the stock assessment. From the present data and under the assumption that the cod used here are representative of cod in the wild, we conclude that such a concern is unsupported and that evasion of ultrasound sources is unlikely to occur.

In addition to echosounders, another anthropogenic ultrasound source is becoming increasingly important in fisheries. Many cetaceans drown by being caught in fishing nets, and such incidents are avoided by equipping the nets with acoustic alarms to deter odontocetes. The pingers used for this emit sounds between 10 and 160 kHz with source levels below 160 dB re. 1 μ Pa (pp)

(Kastelein et al., 2007). Sound sources on fishing nets may, however, also make them detectable to the fish targeted by the nets and in turn lead to reduced catch rates. Although the ultrasonic frequencies are different for these pingers, the lack of behavioural responses at much higher received levels than the source levels of pingers supports the finding by Kastelein et al. (Kastelein et al., 2007) that ultrasonic pingers do not have any effect on Atlantic cod. Finally radio telemetry using high frequency transmitters have been used to measure heart rate on free-swimming Atlantic cod (Claireaux et al., 1995). If ultrasound had an effect on the heart rate and behaviour, results from such experiments would have been compromised by the experimental animals showing abnormal behaviour and heart rates. Present findings do not support concern for such a bias.

Despite the strong negative evidence for ultrasound detection in cod, acoustic stress is nonetheless an important issue to consider in fisheries management and conservation. Atlantic cod has been documented to have acute infrasonic hearing (Sand and Karlsen, 1986) and noise from a research vessel may produce avoidance reactions in Atlantic herring (Ona et al., 2007) and in Atlantic cod (Olsen et al., 1983). Ship noise is, thus, a potential stressor of fish populations and it may introduce a bias in stock assessment and should be investigated further.

If the experimental results on captive cod presented here reflect the behaviour and sensory physiology of wild cod populations, it is concluded that ultrasound exposures mimicking those of echosounders and odontocetes will not induce acute stress responses, such as bradycardia or anti-predator responses in Atlantic cod. Frequent encounters with ultrasound sources will therefore most probably not induce a more chronic state of stress, and thus cause maladies such as increased susceptibility to parasites, abnormal development of larvae and in the end reduced recruitment to the adult population. As the cod used here did not respond to ultrasound, and are not debilitated by very high intensities, the question is which modalities can a cod use to detect an oncoming predator? We consider the low-frequency particle motion created by ships and swimming predators to be likely sensory cues and they should be subjects for further research.

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REFERENCES

- Astrup, J. (1997). Ultrasound detection in fish. Institute of Biology. Aarhus: University of Aarhus.
- Astrup, J. (1999). Ultrasound detection in fish – a parallel to the sonar-mediated detection of bats by ultrasound-sensitive insects? *Comp. Biochem. Physiol.* **124A**, 19-27.
- Astrup, J. and Møhl, B. (1993). Detection of intense ultrasound by the cod *Gadus morhua*. *J. Exp. Biol.* **182**, 71-80.
- Au, W. W. L. (1993). *The Sonar of Dolphins*. New York: Springer Verlag.
- Axelsson, M. (1988). The importance of nervous and humoral mechanisms in the control of cardiac-performance in the Atlantic cod *Gadus morhua* at rest and during non-exhaustive exercise. *J. Exp. Biol.* **137**, 287-301.
- Benoit-Bird, K. J., Au, W. W. L. and Kastelein, R. (2006). Testing the odontocete acoustic prey debilitation hypothesis: no stunning results. *J. Acoust. Soc. Am.* **120**, 1118-1123.
- Claireaux, G., Webber, D. M., Kerr, S. R. and Boutilier, R. G. (1995). Physiology and behavior of free-swimming atlantic cod (*Gadus morhua*) facing fluctuating temperature conditions. *J. Exp. Biol.* **198**, 49-60.
- Clarke, M. R. (1996). Cephalopods as prey. 3. Cetaceans. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **351**, 1053-1065.
- Fordyce, R. E. and Barnes, L. G. (1994). The evolutionary history of whales and dolphins. *Annu. Rev. Earth Planet. Sci.* **22**, 419-455.
- Garrod, D. J. and Schumacher, A. (1994). North Atlantic cod: the broad canvas. *ICES Mar. Sci. Symp.* **198**, pp. 59-76.
- Jónsson, J. (1994). Fisheries off Iceland, 1600-1900. *ICES Mar. Sci. Symp.* **198**, 3-16.
- Kastelein, R. A., van der Heul, S., van der Veen, J., Verboom, W. C., Jennings, N., de Haan, D. and Reijnders, P. J. H. (2007). Effects of acoustic alarms, designed to reduce small cetacean bycatch in gillnet fisheries, on the behaviour of North Sea fish species in a large tank. *Mar. Environ. Res.* **64**, 160-180.
- Kastelein, R., van der Heul, S., Verboom, W. C., Jennings, N., van der Veen, J. and de Haan, D. (2008). Startle response of captive North Sea fish species to underwater tones between 0.1 and 64 kHz. *Mar. Environ. Res.* **65**, 369-377.
- Knudsen, F. R. (1994). Behavioral, physiological and cellular aspects of stress in fish. PhD thesis, Department of Biology, University of Oslo, Norway.
- Lima, S. L. and Dill, L. M. (1990). Behavioral decisions made under the risk of predation – a review and prospectus. *Can. J. Zool.* **68**, 619-640.
- Lombarte, A., Yan, H. Y., Popper, A. N., Chang, J. S. and Platt, C. (1993). Damage and regeneration of hair cell ciliary bundles in a fish ear following treatment with gentamicin. *Hear. Res.* **64**, 166-174.
- Madsen, P. T., Wahlberg, M. and Mohl, B. (2002). Male sperm whale (*Physeter macrocephalus*) acoustics in a high-latitude habitat: implications for echolocation and communication. *Behav. Ecol. Sociobiol.* **53**, 31-41.
- Madsen, P. T., Johnson, M., de Soto, N. A., Zimmer, W. M. X. and Tyack, P. (2005). Biosonar performance of foraging beaked whales (*Mesoplodon densirostris*). *J. Exp. Biol.* **208**, 181-194.
- Mann, D. A., Lu, Z. M., Hastings, M. C. and Popper, A. N. (1998). Detection of ultrasonic tones and simulated dolphin echolocation clicks by a teleost fish, the American shad (*Alosa sapidissima*). *J. Acoust. Soc. Am.* **104**, 562-568.
- Mann, D. A., Higgs, D. M., Tavolga, W. N., Souza, M. J. and Popper, A. N. (2001). Ultrasound detection by clupeiform fishes. *J. Acoust. Soc. Am.* **109**, 3048-3054.
- Michalsen, K., Godo, O. R. and Ferno, A. (1996). Diel variation in the catchability of gadoids and its influence on the reliability of abundance indices. *ICES J. Mar. Sci.* **53**, 389-395.
- Miller, L. A. and Surlykke, A. (2001). How some insects detect and avoid being eaten by bats: tactics and counter-tactics of prey and predator. *Bioscience* **51**, 570-581.
- Miller, L. A., Pristed, J., Mohl, B. and Surlykke, A. (1995). The click-sounds of narwhals (*Monodon monoceros*) in Inglefield Bay, Northwest Greenland. *Mar. Mamm. Sci.* **11**, 491-502.
- Morgan, M. J., Wilson, C. E. and Crim, L. W. (1999). The effect of stress on reproduction in Atlantic cod. *J. Fish Biol.* **54**, 477-488.
- Norris, K. S. and Møhl, B. (1983). Can odontocetes debilitate prey with sound. *Am. Nat.* **122**, 83-104.
- Oiestad, V. (1994). Historic changes in cod stock and cod fisheries: Northeast Arctic cod. *ICES Mar. Sci. Symp.* **198**, 17-30.
- Olsen, K., Angell, J., Pettersen, F. and Løvik, A. (1983). Observed fish reactions to a surveying vessel with special reference to herring, cod, capelin and polar cod. *FOA Fish. Rep.* **300**, 131-138.
- Ona, E., Godo, O. R., Handegard, N. O., Hjelvik, V., Patel, R. and Pedersen, G. (2007). Silent research vessels are not quiet. *J. Acoust. Soc. Am.* **121**, EL145-EL150.
- Pickering, A. D. and Pottinger, T. G. (1989). Stress responses and disease resistance in salmonid fish – effects of chronic elevation of plasma-cortisol. *Fish Physiol. Biochem.* **7**, 253-258.
- Plachta, D. T. T. and Popper, A. N. (2003). Evasive responses of American shad (*Alosa sapidissima*) to ultrasonic stimuli. *Acoust. Res. Lett. Online* **4**, 25-30.
- Remage-Healey, L., Nowacek, D. P. and Bass, A. H. (2006). Dolphin foraging sounds suppress calling and elevate stress hormone levels in a prey species, the Gulf toadfish. *J. Exp. Biol.* **209**, 4444-4451.
- Ripple, W. J. and Beschta, R. L. (2004). Wolves and the ecology of fear: can predation risk structure ecosystems? *Bioscience* **54**, 755-766.
- Sand, O. (1984). Lateral-line systems. In *Comparative Physiology of Sensory Systems* (ed. L. Bolis, R. D. Keynes and S. H. P. Maddrell), pp. 3-32. Cambridge: Cambridge University Press.
- Sand, O. and Karlsen, H. E. (1986). Detection of infrasound by the Atlantic cod. *J. Exp. Biol.* **125**, 197-204.
- Santos, M. B., Pierce, G. J., Reid, R. J., Patterson, I. A. P., Ross, H. M. and Mente, E. (2001). Stomach contents of bottlenose dolphins (*Tursiops truncatus*) in Scottish waters. *J. Mar. Biol. Assoc. U.K.* **81**, 873-878.
- Schreck, C. B. (1996). Immunomodulation: endogenous factors. In *The Fish Immune System: Organism, Pathogen, and Environment*. Vol. 15 (ed. G. Iwama and T. Nakanishi), pp. 311-338. London: Academic Press.
- Simmonds, J. and MacLennan, D. (2005). *Fisheries Acoustics, Theory and Practice*. Oxford: Blackwell Science.
- Sokal, R. R. and Rohlf, F. J. (1998). *Biometry: the principles and practice of statistics in biological research*. New York: W. H. Freeman.
- Sokolov, E. N. (1963). Higher nervous functions – orienting reflex. *Annu. Rev. Physiol.* **25**, 545-580.
- Urick, R. J. (1983). *Principles of Underwater Sound*. Los Altos: Peninsula Publishing.
- Wilson, M., Hanlon, R. T., Tyack, P. L. and Madsen, P. T. (2007). Intense ultrasonic clicks from echolocating toothed whales do not elicit anti-predator responses or debilitate the squid *Loligo pealeii*. *Biol. Lett.* **3**, 225-227.
- Wilson, M., Acolas, M.-L., Bégout, M.-L., Madsen, P. T. and Wahlberg, M. (2008). Ultrasound detection and intensity-graded anti-predator response in Allis shad (*Alosa alosa*). *JASA Expr. Lett.* (in press).
- Zagaeski, M. (1987). Some observations on the prey stunning hypothesis. *Mar. Mamm. Sci.* **3**, 275-279.