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**Onderzoek naar de invloed van onderwatergeluid op vissoorten van de Noordzee**

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**Reactions of North Sea fish species to underwater sounds in a wide frequency range**

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## Samenvatting

Wereldwijd neemt het antropogeen veroorzaakte onderwatergeluid toe. Om de potentiële effecten van antropogeen geluid op zeevis te kunnen voorspellen is informatie nodig over het gehoor van vis. Echter, als een vis een geluid detecteert, betekent dat niet meteen dat deze er een reactie op zal vertonen. Bij de meeste dieren moet het geluid boven een bepaald niveau komen voordat het gedrag wordt beïnvloed. In deze studie werden dergelijke geluidsniveaudrempels vastgesteld voor acht vissoorten die voorkomen in de Noordzee: zeebaars, diklipharder, steenbolk, kabeljauw, paling, pollak, horsmakreel en haring. Deze vissoorten werden getest in scholen van één soort. Hun reacties op tonen in het frequentiegebied van 100 Hz tot 64 kHz en op één breedbandig ruissignaal werden geobserveerd. Per signaaltype werden drie niveaustappen vastgesteld (geen reactie, soms reactie, meestal reactie), of het maximale niveau dat met de beschikbare apparatuur voor een bepaalde toonhoogte kon worden geproduceerd. Elke frequentie/niveau combinatie werd per vissoort 12 (pollak 18) maal getest, over een periode van ongeveer 10 dagen. Op basis van deze gegevens werd voor elke vissoort per frequentie het 50 % reactiedrempel-geluidsniveau in een psychometrische functie bepaald. Gedragsparameters die duidelijk een verandering vertoonden waren zwemsnelheid, zwemrichting en lichaamshouding.

Voor de zeebaars werden de 50 % reactiedrempel-geluidsniveaus bereikt voor stimuli tussen 100 Hz and 700 Hz, voor de diklipharder tussen 400 Hz en 700 Hz, voor de steenbolk tussen 100 Hz and 250 Hz, voor de horsmakreel tussen 100 Hz en 2000 Hz, en voor de Atlantische haring voor 4000 Hz signalen. De dieren reageerden niet op de maximale geluidsniveaus die konden worden geproduceerd voor hogere frequenties. De reactiedrempel-geluidsniveaus (ontvangen door de vis) namen ruwweg toe van rond 100 dB (re 1  $\mu$ Pa) bij 100 Hz tot rond 160 dB bij 700 Hz. Voor kabeljauw, pollack en paling konden geen 50 % reactiedrempels worden bereikt voor de testfrequenties en het breedbandige ruissignaal. Alleen de steenbolk en horsmakreel reageerden op het breedbandige ruissignaal.

Deze studie toont aan dat het verschil tussen de gehoordrempel en de reactiedrempel verschilt per frequentie binnen een vissoort en tussen vissoorten. Dit suggereert dat in de zee, niet alleen het maskerende effect van het achtergrondgeluid bepaald of een geluidssignaal een effect heeft op visgedrag, maar ook de frequentie/geluidsniveau-combinatie van het signaal. Bovendien toont de huidige studie aan dat vissoorten erg verschillend reageren op geluid, en dat algemene opmerkingen over effecten van geluid op vis niet nuttig zijn zonder de vissoort en de geluidsparameters te specificeren.

Behalve haring reageerden de meeste vissoorten op geluiden met frequenties onder de 1000 Hz. Over het algemeen hebben antropogene geluidsbronnen op zee de meeste energie in het laagfrequente gebied (< 1 kHz). Bovendien draagt laagfrequent geluid verder dan hoogfrequent geluid, omdat het minder verzwakt over afstand. Daarom zal vis, zeer waarschijnlijk, worden beïnvloed door menselijke activiteiten op zee indien de geluidsniveaus hiervan boven de reactiedrempel-geluidsniveaus komen, die vastgesteld zijn in de huidige studie (mogelijke gewenning aan geluid is in deze studie niet aan de orde geweest).

De beperking van de huidige studie is dat slechts acht van de 160 vissoorten die voorkomen in de Noordzee, zijn onderzocht. Omdat er al binnen deze acht soorten vrij grote verschillen in reactiedrempel-geluidsniveaus en in toonhoogten waarop de dieren reageerden optreden, is het belangrijk om meer vissoorten te testen, om zo beter te kunnen voorspellen hoe vis in Noordzee zal reageren op antropogeen onderwatergeluid.

Met additionele apparatuur is het mogelijk om de onder- en bovengrens van het frequentiegebied nauwkeuriger vast te stellen. In de huidige studie is de reactie van vis op tonen and breedbandige ruis bestudeerd. Het is zeer zinvol om ook de reactie van vis op meer gecompliceerde geluiden (b.v. concrete antropogene geluiden) te onderzoeken.

## Abstract

World-wide, underwater anthropogenic noise is increasing. To predict potential effects of man-made noise on marine fish, information is needed on the hearing sensitivity of fish for certain types of sounds. However, when a fish detects a sound, this does not mean that it will react to it. In most animals, sound needs to reach a certain sound pressure level before the behaviour of an animal is affected. In this study such threshold levels were attempted to be determined for eight fish species occurring in the North Sea: sea bass (*Dicentrarchus labrax*), thicklip mullet (*Chelon labrosus*), pout (*Trisopterus luscus*), cod (*Gadus morhua*), eel (*Anguilla anguilla*), pollack (*Pollachius pollachius*), horse mackerel (*Trachurus trachurus*) and Atlantic herring (*Clupea harengus*). The fish were housed as single-species schools in a tank. Their reactions to pure tones in the frequency range between 100 Hz and 64 kHz and to one type of broadband noise were observed. Per frequency, three levels were determined (no reaction, sometimes a reaction, usually a reaction), or in some cases only the maximum level that could be produced for a particular frequency with the available equipment was tested. Each frequency/level combination was tested 12 times per fish species (18 for pollack) over a period of about 10 days. Based on these results, per frequency, the 50 % reaction threshold level in a psychometric function was determined per fish species. The behavioural parameters that clearly showed a reaction to the sound stimuli were changes in swim speed, swim direction and body shape.

For sea bass the 50 % reaction thresholds were reached for signals between 100 Hz and 700 Hz, for the thicklip mullet between 400 Hz and 700 Hz, for pout between 100 Hz and 250 Hz, for horse mackerel between 100 Hz and 2000 Hz and for Atlantic herring at 4000 Hz. The reaction threshold exposure levels increased generally from around 100 dB (re 1  $\mu$ Pa) at 100 Hz to around 160 dB at 700 Hz. For cod, pollack and eel no 50 % reaction thresholds were reached for any of the test frequencies and the broadband noise signal. Only Pout and horse mackerel reacted to the broadband noise stimulus.

The present study shows that the difference between the hearing and reaction threshold levels varies per frequency within a species and between species. This suggests that at sea, not only the masking effect of the ambient noise on a stimulus determines its effect on fish behaviour, but also the frequency/level combination of a signal. In addition the present study shows that fish species react very differently to sound, and that general remarks on effects of sound on fish are not very useful without specifying the fish species and the sound characteristics.

Except for herring, most of fish reacted to sounds below 1000 Hz. In general most of the energy of anthropogenic noise sources at sea is low-frequency (< 1 kHz). In addition, low-frequency sounds travel far, as they attenuate less over distance than high-frequency sounds. Therefore fish are likely to be influenced by anthropogenic activities if the exposure level is above the reaction threshold level determined in the present study (potential habituation to these sounds was not studied).

The limitation of the present study is that only eight of the 160 fish species that occur in the North Sea, were tested. Because already within the eight species marked differences in reaction threshold levels and in frequencies, which caused reaction, were observed, it seems important to conduct the same test on more fish species, to be able to better predict the potential reaction of fish of the North Sea to anthropogenic underwater noise.

With special equipment, the upper and lower frequency limits to which the fish species react can be determined more accurately. The present study tested the animals' reaction to tones and one type of broadband noise. It is of interest to test the animals' reaction to more

complicated sounds such as actual man-made noise (shipping and wind turbine noise for instance).

## Introduction

World-wide, underwater background noise levels are increasing due to anthropogenic activities. Many marine organisms rely heavily on acoustics to survive. Fish for instance have very complex and diverse relationships with sound and acoustic energy. Fish use sound to engage with their surroundings, by using acoustic adaptations particular to their species – for hunting, territorial behaviour, bonding, spatial orientation, predator aversion, etc. Such ecologically important behaviours can be negatively influenced by anthropogenic noise. Little is known about the effects of anthropogenic noise on marine fish and to reliably predict the potential effects of certain man-made sounds on marine fish much information is needed.

The effect of a sound may depend on: 1) properties of the sound, such as frequency spectrum, source level (SL), duration, rise and fall times in level, and repetition rate, 2) background noise (masking), 3) sound level and spectrum received by the animal (exposure level), 4) exposure duration, 5) hearing properties of the species (sensitivity, directivity index and critical ratio), and 6) species-specific or individual reactions to sound.

Little information is available on the hearing sensitivity of marine fish. Such information exists for only a few species. Most audiograms of marine fish species indicate their highest sensitivity to sounds within the 100 Hz – 2 kHz range. This narrow bandwidth of hearing sensitivity could be due to mechanical limitations of the sense organs, or physical constraints of the testing systems (**Table 1**). However, recent studies have shown that Clupeid fish may also be able to hear ultrasound (Mann *et al.*, 1997, 1998, 2001, 2002) although Pacific herring cannot detect ultrasound (Mann *et al.*, 2005).

When a fish can detect a sound, this does not mean that it will react to it. Some studies have investigated the effects of specific sounds on the behaviour of some marine fish species (**Table 2**). In most animals, sound needs to reach a certain sound pressure level before the behaviour of an animal is affected. The aim of the present study was to determine the reaction threshold levels of eight fish species from the North Sea to pure tones in the frequency range between 100 Hz and 64 kHz and to one type of broadband noise.

## Materials and Methods

### *Study animals*

Eight fish species that are found in the North Sea were selected for testing, based on their availability, their ease of maintenance in captivity, the temperatures at which they can be kept (the water temperature in the tank was influenced by the environment), and their economic importance in fisheries. The animal welfare commission of the Netherlands stipulated that the fish used must feed readily in captivity, so they had to come from aquaria or fish farms, though most were originally wild-caught.

The study fish species, sea bass (*Dicentrarchus labrax*), thicklip mullet (*Chelon labrosus*), pout (*Trisopterus luscus*), cod (*Gadus morhua*), pollack (*Pollachius pollachius*) and horse mackerel (*Trachurus trachurus*) were borrowed from “The Arsenaal Aquarium”, Vlissingen (**Table 3**). The fish had been wild-caught by hook and line or in a trap, so that no obvious damage had occurred to their swim bladder, which is used in hearing in many fish species. The Eel (*Anguilla anguilla*) came from “Schot aquacultuur”, Bruinisse. The Atlantic

herring (*Clupea harengus*) were borrowed from the Oceanium department of the Blijdorp Zoo, Rotterdam. The fish used in this study were all adapted to captivity and were feeding voluntarily.

Except for herring, the animals were fed *ad lib.* pieces of raw fish (food was given until the animals stopped eating) twice a week after the daily study sessions. The herring were fed Trouvit pellets size no. 00 (Nutreco Aquaculture) from a food dispenser throughout the day. The amount eaten was related to the water temperature. Some days before each species was tested, the fish were kept in white polyester 2.2 m diameter holding tanks with a water depth of 1 m. In those tanks, most fish swam slowly or remained stationary most of the time. During the study the species were kept in a large tank in schools of 4-17 individuals.

### *Study area*

The experiments were conducted in an outdoor tank at the Oosterschelde Research Center for Aquatic studies (ORCA) in Wilhelminadorp, Zeeland, The Netherlands. The rectangular tank (7.0 m long, 4.0 m wide; water depth 2.0 m) was made of plywood covered on both sides with fibreglass (**Fig. 1**). The tank was placed into a 1 m deep hole in the ground. The tank sat on a layer of rubber tiles, and the parts of the sides below ground level were covered with a layer of 3 cm thick Styrofoam to reduce contact noise from the environment in the pool. The pool walls and floor were blue (Ral colour 50/15).

To reduce predation by birds, algal growth, impact of noise from rain, glistening of the water surface, and to create a more even light pattern in the pool, a slanting roof was build above the pool in the form of a car port (2.5 m on one side and 2.0 m on the other side).

The water was pumped directly from the nearby Oosterschelde (a lagoon of the North Sea). The salinity was 30- 33 ‰. To ensure the good water clarity needed to film the fish, the water was circulated via sand, UV light, and carbon filters. During the experiments the water system was a closed circuit for the period in which each fish species was tested. Water temperature was measured daily and remained well within the boundaries suitable for the fish species tested (**Table 4**).

To make the environment inside the tank as quiet as possible, the filter unit had a low noise “whisper” pump. To reduce contact noise entering the pool, the pump and filter unit were placed on rubber tiles like the pool. To reduce contact noise further, the filtration pump was connected to the tank with flexible tubes.

To ensure that during test sessions all fish could be filmed at each particular moment with one or more of the three cameras, and to make a change in fish species easy and animal friendly, the fish were kept in a net enclosure (4 m long, 1.9 m wide and 2.5 m high) that was rigged over the width of the tank (**Fig. 1**). The net was made of white nylon, 1.5 cm stretched mesh). By means of lead lines and four weights in the corners, the enclosure kept its rectangular shape.

Two research cabins were placed on one side of the tank. One housed the sound generation equipment, three monitors, video recording equipment, and sound recording equipment. The other cabin housed the sound calibration equipment.

Between October and December, artificial lighting was used during the first session of the day.

### *Stimuli*

The fish were subjected to two types of stimuli: pure tones and broadband noise. Pure tones of the following frequencies were tested: 100 Hz, 125 Hz, 250 Hz, 400 Hz, 500 Hz, 600 Hz, 700 Hz, 800 Hz, 900 Hz, 1 kHz, 2 kHz, 4 kHz, 8 kHz, 16 kHz, 32 kHz, 45 kHz, and 64 kHz. The

broadband noise signal was intended to be white noise, but due to the characteristics of the transducer and tank, the energy varied considerably between frequencies (see Fig. 3- 10). The stationary portion of the signal was 900 ms in duration. Rise and fall times (each 50 ms) preceded and followed the signal to prevent abrupt signal onset and offset transients.

The sounds were produced by a generator (Hewlett Packard, model 33120A), a signal shaper and attenuator (a modified audiometer, Midimate model 602, s/n 29433; 5-dB steps), a power amplifier (HQ Power, model VPA2200BMN-2 x 200 Wrms), and three underwater transducers, depending on the frequency of the projected sounds:

1) For signals between 100 Hz - 250 Hz, an Ocean Engineering Enterprise transducer, model DRS-12; 30 cm diameter and its impedance matching transformer;

2) For signals between 400 Hz - 45 kHz, an Ocean Engineering Enterprise transducer, model DRS-8; 20 cm diameter and its impedance matching transformer (this transducer was also used to produce the broadband noise signal);

3) For 64 kHz signals, an Airmar high frequency transducer.

During a pre-test with each fish species, the signal levels for the main study were determined by increasing the sound pressure level of each frequency (and noise signal) until a reaction to the stimulus was observed (this response can be best described as a startle response). That level was tested, as well as a 5 dB higher and lower level. Some signal frequencies (and noise signal) caused no reaction when produced at the highest sound pressure level that could be generated with the available equipment. In such case, that maximum producible level was tested during the main experiment.

During test sessions the audible stimuli and background noise were checked with a hydrophone (Labforce 1 BV, model 90.02.01), a charge amplifier (Brüel & Kjaer (B&K), model 2635) and an amplified loudspeaker box. For sounds above 16 kHz, the loudspeaker box was replaced by a heterodyne frequency reducer (Stag Electronics, UK, model Batbox III). The outputs of the charge amplifier and frequency reducer were fed into the video recorders (via ground loop isolators), so that the fish' behaviour around the stimulus presentation could later be analysed.

#### *Sound parameters and sound distribution in tank*

Two types of sound measurement were carried out during the experiments: 1) determination of the background noise in the pool, to check whether the stimulus sounds were not masked by background noise; 2) determination of the sound pressure levels (SPLs) at two locations in the net enclosure during sound emissions, to check the distribution of the stimulus sounds in the study area.

The equipment used to measure background noise and stimulus SPLs (up to 45 kHz) was the same and consisted of a broadband hydrophone (B&K 8101, 0-100 kHz), a voltage amplifier system (TNO TPD, 0-300 kHz) and a personal computer with spectral analysis software (Cool Edit Pro, Syntrillium Software Corp., USA; sample frequency 11-96 kHz, frequency range 0-48 kHz,  $df = 15-115$  Hz). The total system was calibrated with a pistonphone (B&K 4223) and a white noise 'insert voltage signal' into the hydrophone pre-amplifier. Measurements were corrected for the frequency sensitivity of the hydrophone and the frequency response of the measurement equipment.

The 64 kHz signal was calibrated with a calibrated hydrophone (RESON, TC 4032, S/N 1704048), connected (20 m extension cable) to a RESON EC 6073 input module, which facilitated as splitter for signal transfer to a computer and the powering of the hydrophone with a DC supply battery PBQ 17 of 12.6 V/17Ah. An ETEC A1101 battery powered amplifier was used to condition the hydrophone signal with a gain of 10-20 dB (selectable between 0-50 dB) as well as high pass filter. In this set-up a low cut setting of 10 Hz was

selected to reduce the self-noise of the hydrophone. As the gain characteristics are flat to 1 MHz, a low pass filter was used on the output of the amplifier to filter the HF noise above 150 kHz with 12 dB/octave. The output of the filter was connected via a BNC 2110 coaxial input module to a 16 bit data acquisition card (National Instruments type PCI 6281M) on which the analogue signals were digitized with a sample rate of 512 kHz. Of each data sample the SPL (Sound Pressure Level) was computed using the SPL/voltage relation of a pistonphone (G.R.A.S., model 42AC) reference source and a B&K 2239 sound level meter to measure the SPL reduction with the hydrophone coupled into the pistonphone. With this reference all system errors in the analogue/digital link were eliminated, assuming a flat response curve of the hydrophone up to 100 kHz. Above this range the fall-off characteristics will be incorporated in the final calibration. The computer with the DAQ card was powered via an UPS (APC 1400) to maintain a floating earth circuit uncoupled from the local earth system. The data monitoring/acquisition/analysis functions were conducted using special RIVO-developed acoustic software modules, build with Labview 7.0 software (National Instruments). The spectrograms were computed in narrow-band FFT. Highest noise immunity was obtained when the input module housing was connected to the housing of the BNC 2110 BNC input module and the ground terminal of the AC mains floating and with system earth was terminated to the basin water.

Background noise levels were determined in the range 20 Hz - 48 kHz and the narrow-band Fast Fourier Transform (FFT) results were converted to Power Spectral Density (PSD) levels (1 Hz bandwidth) and time-averaged over 32 s (**Fig. 2**). Due to the absence of important mechanical sources, background levels were very low (below sea state 1; Wentz, 1962). Only in the range below 100 Hz levels are somewhat higher.

Stimulus sound levels were measured four times well distributed over the study period in the area in which the fish usually swam, 0.5 m above the bottom in the center line of the net enclosure, at a distance of 1.5 and 3.5 m from the sound sources (transducers). Two frequency ranges were applied to measure the sound distribution in the pool: 20-500 Hz (sample frequency 11.025 Hz) and 0.4-48 kHz (sample frequency 96 kHz). For each stimulus frequency the spectra of three sound blocks (900 ms duration each) were determined and averaged. Due the fact that for pure tones the pool was reverberant (standing waves) the propagation loss fluctuated considerably and deflected from the '20 log R' attenuation law. In the net the stimuli levels varied at most by  $\pm 8$  dB from the average level. This level range has thus been used to show the average 50 % reaction threshold exposure levels. During the measurements it was checked whether the sounds contained harmonic components.

### *Observation equipment*

The behaviour of the fish was recorded by three black and white underwater video cameras (Mariscope, model Micro, Kiel, Germany). The animals were filmed from above. The cameras were mounted in a row across the width of the pool (**Fig. 1**), with the lens just below the water surface so that about 80 % of the water volume in the net enclosure was in view. Just below the water surface some parts were not in view, but those were never used by the fish species tested, as they swam closer to the bottom. The images of the three cameras were matching; there was no overlap.

### *Methodology*

In each test a school of fish of only one fish species was used, in order to avoid the chance of the behaviour of one species influencing the behaviour of another. The 4 - 17 fish of each species were placed in the tank at least a day before the first session with that species was



conducted. This allowed the fish to habituate to the tank. The transducers and cameras were placed in the pool at the beginning of each working day and remained in the water during all sessions. Each one hour session consisted of ten 1-minute recordings during which a sound was projected 30 s after the onset of the trial. The time between trials was 5 minutes. This inter trial time was based on a pre-test in which the inter trial time of a signal, at a particular level which caused a startle response when first projected, was reduced from 10 minutes to 1 minute. Often the fish did not react when successive signals occurred with one minute in between, but response was restored after two minutes. Therefore a “safe” (conservative) inter trial time of five minutes was chosen for the main experiment.

As the pump in the pool was extremely quiet and connected to the pool with rubber hoses, it was left on during the experiments, so as not to change the background noise before and during the sessions.

Usually four sessions of 60 minutes each were conducted daily between at 08.30 and 16.00 hrs. Per fish species, all frequency/level combinations, determined during the pre-test, were offered in a random order during the approximately ten day study period of that species. Per fish species, each frequency/level combination was tested 12 times (pollack 17 times). The study was conducted between October 2004 and December 2005.

### *Analysis*

The data collection and analysis was done by two researchers. During the actual stimulus projection the operator, which could see the entire study area on three monitors in the research cabin, recorded whether the fish (general impression of the group in view) reacted to a particular stimulus or not. After each session, the recordings of the three cameras were analysed by the other researcher. Each tape was analysed, and the reaction of each fish in the school was recorded.

A reaction to a stimulus was judged by a sudden change in swim speed, swim direction or body posture. If more than 30 % of the school reacted to the stimulus, the trial was classified as a “reaction” trial. The two researchers alternated tasks between sessions, and when analysing the video recordings, were not aware of the other person’s classification of the trials during the actual sessions.

Per signal frequency/level combination, the % of the 12 (17 pollack) trials the fish reacted to was calculated. Based on these percentages psychometric curves were drawn (exposure level versus % reaction). From these curves, the 50 % reaction threshold sound pressure levels were derived. Those levels were used to draw the reaction threshold curve for each species.

## **Results**

### Sea bass (*Dicentrarchus labrax*)

Sea bass was relatively responsive to sound and 50 % reaction thresholds were reached for signals between 100 Hz and 700 Hz (**Fig. 3**). The animals did not react to the maximum exposure level that could be produced for the higher frequency signals and the broadband noise signal. The 0% reaction threshold exposure levels were about 8 dB below the 50 % reaction threshold levels.

### Thicklip mullet (*Chelon labrosus*)

For the thicklip mullet, 50 % reaction thresholds were reached for signals between 400 Hz and 700 Hz (**Fig. 4**). The animals did not react to the maximum producible exposure levels that could be produced for the other frequencies and the broadband noise signal. However, the fish reacted to some of the 100 and 125 Hz signals (10-16 % of the trials), which suggests that the 50 % reaction threshold level for those frequencies was only a few dB above the maximum level that could be produced with the equipment. The 0% reaction threshold exposure levels were about 8 dB below the 50 % reaction threshold levels.

#### Pout (*Trisopterus luscus*)

Pout was relatively medium responsive to sound and 50 % reaction thresholds were reached for signals between 100 Hz and 250 Hz (**Fig. 5**). The animals did not react to the maximum exposure level that could be produced for higher frequencies. However, the pout did react to the broadband noise signal, and the 50 % reaction threshold for this noise could be calculated. The 0% reaction threshold exposure levels were about 8 dB below the 50 % reaction threshold levels.

#### Cod (*Gadus morhua*)

Cod was relatively unresponsive to sound, and no 50% reaction thresholds were reached for any of the tested frequencies and the broadband noise signal (**Fig. 6**).

#### Eel (*Anguilla anguilla*)

Eel was relatively unresponsive to sound, and no 50 % reaction thresholds were reached for any of the tested frequencies and the broadband noise signal (**Fig. 7**)

#### Pollack (*Pollachius pollachius*)

Pollack was relatively unresponsive to sound, and no 50 % reaction thresholds were reached for any of the test frequencies and the broadband noise signal (**Fig. 8**). There was some reaction between 100 Hz and 300 Hz.

#### Horse mackerel (*Trachurus trachurus*)

The horse mackerel was relatively responsive to sound and 50 % reaction thresholds were reached for signals between 100 and 2000 Hz (**Fig. 9**). The animals did not react to the maximum exposure level that could be produced for the higher frequencies. The horse mackerel did react to the noise stimulus. The 0% reaction threshold exposure levels were about 8 dB below the 50 % reaction threshold levels.

#### Atlantic herring (*Clupea harengus*)

Atlantic herring reacted to two frequencies. The 50 % reaction threshold was reached for only the 4000 Hz signal. There was also some reaction to 400 Hz signals (**Fig. 10**). The animals did not react to the maximum exposure level that could be produced for the other frequencies and the broadband noise signal. The 0% reaction threshold exposure level at 4000 kHz was 10 dB below the 50 % reaction threshold level.

## Discussion and Conclusions

### *Observations*

The size of a tank has an influence on the general swimming behaviour of many fish species. Before the fish were put in the test tank, they were kept in much smaller circular tanks, in which they swam very slowly or not at all. In the large test tank, the fish were much more active. So, although the test tank was far from a natural environment, it may be a much better study area than the smaller tanks that have been used in several previous studies on fish reaction to sound.

### *Differences between 0 % reaction level and 50 % reaction level*

The differences between 0 % reaction SPL and 50 % reaction SPL was on average 8 dB and was thus similar to the lowest level of the 50 % exposure threshold level range.

### *Differences in reaction to the stimuli between fish species*

Of only four of the tested fish species the hearing sensitivity has been tested, either physiologically or behaviourally (see fish audiograms in Nedwell *et al.*, 2004). For those species it can be stated that the background noise level in the tank was so low that it did not mask the test stimuli (**Fig. 2**).

In the sea bass, the 50 % reaction threshold levels were 10-30 dB above the sea bass' hearing thresholds for the test frequencies (ABR method, Lovell, 2003, in: Nedwell *et al.*, 2004; **Fig. 3**). In the cod, the 50 % reaction threshold levels were not even reached when the test signals were 15-40 dB above the cod's hearing thresholds for those frequencies obtained by Buerkle (1967; **Fig. 6**), and 40-60 dB above the hearing thresholds obtained for the same species by Chapman & Hawkins (1973; **Fig. 6**). In the pollack, the 50 % reaction threshold levels were not even reached when the test signals were 30-50 dB above the hearing thresholds for the test frequencies obtained by Chapman & Hawkins (1969; **Fig. 8**). In herring, the 50 % reaction threshold level was 30 dB above the herring's hearing threshold at 4 kHz (Enger, 1967; **Fig. 10**).

Thus, the present study shows that the difference between the hearing and reaction threshold levels varies per frequency within a species and between species. This suggests that at sea, not only the masking effect of the ambient noise on a stimulus determines its effect on fish behaviour, but also the frequency/level combination of a signal. In addition the present study shows that fish species react very differently to sound, and that general remarks on effects of sound on fish are not very useful without specifying the fish species and the sound characteristics.

Although the broadband noise spectral level was probably below their hearing threshold levels, and the 50 % reaction threshold levels found in the present study, pout and horse mackerel did react to a broadband noise level that was 5 dB lower than the maximum producible level. This suggests that the energy in certain frequency bands is added by the hearing system of these fish species.

### *Results in relation to anthropogenic noise*

Except for herring, the fish species which showed reactions to the producible sounds reacted to sounds below 1000 Hz. In general anthropogenic noise sources have their maximum energy below 1 kHz (Richardson *et al.* 1995). In addition, low-frequency sound travels far, as it

attenuates less over distance than high-frequency sound. Therefore fish are likely to be influenced by anthropogenic activities if the acoustic exposure level is above the reaction threshold levels determined in the present study. The exposure level depends on, among other parameters, the source level of the sound source and the distance between the sound source and the fish (propagation loss). Potential habituation to sounds has not been investigated in the present study.

#### *Suggestions for further research*

The limitation of the present study is that only eight of the 160 fish species that occur in the North Sea, were tested. Because already within the eight species marked differences in reaction threshold exposure levels and in frequencies which caused reaction, were observed, it seems important to conduct the same test on more fish species, to be able to better predict the potential reaction of marine fish of the North Sea to anthropogenic noise.

With additional equipment, the upper and lower frequency limits (below 100 Hz and above 1 kHz) to which the fish species react can be determined more accurately. The present study tested the animals' reaction to tones and one broadband noise. It is of interest to test the animals' reaction to more complicated sounds such as actual anthropogenic noise, for instance the noise of wind turbines and shipping.

For fish species of commercial, scientific or public interest, audiograms could be obtained in follow-up studies.

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**Tables:**

Table 1. The marine fish species of which the hearing sensitivity has been measured. Note that in many cases only detection of low frequency sounds were tested, so the resulting detected frequency range of hearing may not be the entire hearing range of the species.

<b>Fish species</b>	<b>Latin name</b>	<b>Frequency range tested</b>	<b>Detected frequency range of hearing</b>	<b>Method</b>	<b>Source</b>
<b>Roundfish</b>					
Cod	<i>Gadus morhua</i>	200 –800 Hz tones. Signal duration and interval unknown	200 Hz-800 Hz	Electro-physiological	Enger and Andersen, 1967
Cod	<i>Gadus morhua</i>	30-470 Hz	60-310 Hz	Heart rate	Chapman and Hawkins, 1973
Cod	<i>Gadus morhua</i>	75 Hz		Direction finding	Schuijf and Buwalda, 1975
Cod	<i>Gadus morhua</i>	110 Hz, 8 s tone pulse	Directional hearing	Heart rate	Hawkins and Sand, 1977
Cod	<i>Gadus morhua</i>	??	??	??	Sand and Karlsen, 1986
Cod	<i>Gadus morhua</i>	38 kHz pulses of 3 ms	38 kHz at 194 dB		Astrup and Møhl, 1993
Cod	<i>Gadus morhua</i>	Difference between low and high rep. rates		Heart rate	Astrup and Møhl, 1998
American shad	<i>Alosa sapidissima</i>	0.2-180 kHz pure tones	200-800 Hz and 25-130 kHz	Heart rate	Mann <i>et al.</i> , 1997, 1998
American shad	<i>Alosa sapidissima</i>	20 ms tones every 9 s. 600 Hz and 40, 60 and 80 kHz	600 Hz-80 kHz	Auditory Brainstem Response (ABR)	Mann <i>et al.</i> , 2001
Atlantic Herring	<i>Clupea harengus</i>	30-4000 Hz	30-1200 Hz	ABR	Enger, 1967
Gulf menhaden	<i>Brevoortia patronus</i>	20 ms tones every 9 s. 600 Hz and 40, 60 and 80 kHz	600 Hz- 80 kHz	ABR	Mann <i>et al.</i> , 2001
Spanish sardine	<i>Sardinella aurita</i>	20 ms tones every 9 s. 600 Hz and 40, 60 and 80 kHz	600 Hz to 4 kHz	ABR	Mann <i>et al.</i> , 2001

Table 2. Continued.

<b>Fish species</b>	<b>Latin name</b>	<b>Frequency range tested</b>	<b>Detected frequency range of hearing</b>	<b>Method</b>	<b>Source</b>
Scaled sardine	<i>Harengula jaguana</i>	20 ms tones every 9 s. 600 Hz and 40, 60 and 80 kHz	600 Hz to 4 kHz	ABR	Mann <i>et al.</i> , 2001
Spotlined sardine	<i>Sardinops melanostictus</i>	500-2000 Hz	700-1100	ABR	Akamatsu <i>et al.</i> , 2003
Bay anchovy	<i>Anchoa mitchilli</i>	20 ms tones every 9 s. 600 Hz and 40, 60 and 80 kHz	600 Hz to 4 kHz	ABR	Mann <i>et al.</i> , 2001
Japanese anchovy	<i>Engraulis japonicus</i>	100-700 Hz	200-400 Hz	Behaviour	Akamatsu <i>et al.</i> , 1996
Sculpin	<i>Cottus scorpius</i>	200–800 Hz tones. Signal duration and interval unknown.	No reaction. No swim bladder	Electro-physiological	Enger and Andersen, 1967
Atlantic salmon	<i>Salmo salar</i>	25-580 Hz	< 380 Hz	Cardiac conditioning. Studies in river and laboratory.	Hawkins and Johnstone, 1978
Yellowfin tuna	<i>Thunnus albacares</i>	50-1500 Hz	300-500 Hz	Behavioural (conditioned)	Iversen, 1967
Kawakawa	<i>Euthynnus affinis</i>	100-1100 Hz	300-800 Hz	Behavioural	Iversen, 1969
Red Sea Bream	<i>Pagrus major</i>	50-1000 Hz	100-300 Hz	Heart rate conditioning	Ishioka <i>et al.</i> , 1988
Black rockfish	<i>Sebastes schlegeli</i>	100-500 Hz	300-500 Hz	Basic and masked audiogram. Heart rate conditioning	Motomatsu <i>et al.</i> , 1998
<b>Flatfish</b>					
Flounder	<i>Platichthys flesus</i>	200-800 Hz	300 Hz	Behavioural conditioning	Anraku <i>et al.</i> , 1998
Plaice & Common dab	<i>Pleuronectus platessa</i> & <i>Limanda limanda</i>	25-300 Hz	110-160 Hz	Cardiac conditioning	Chapman and Sand, 1974
Plaice	<i>Pleuronectus platessa</i>	0.1 – 30 Hz (infrasound)	All signals	Cardiac conditioning	Karlsen, 1992
Bastard halibut	<i>Paralichthys olivaceous</i>	100-1600 Hz	200-340 Hz	Heart rate conditioning	Fujieda <i>et al.</i> , 1996 Fujieda, 1998
<b>Sharks</b>					
Horn shark	<i>Heterodontus francisci</i>	25-160 Hz	40 Hz	Conditioned behaviour	Kelly and Nelson, 1975
Lemon shark	<i>Negaprion brevirostris</i>	??	??	??	Nelson, 1967

Table 2. Studies on the effects of sound on the behaviour of marine fish.

Fish species	Latin name	No. of animals	Frequency spectrum (kHz)	SPL (dB re 1 $\mu$ Pa @ 1m)	Signal duration (ms)	Inter signal time (s)	Exposure time (min)	Reaction	Reference source
Blueback herring	<i>Alosa aestivalis</i>	50-100	0.1-1 & 80-420 Pure tones	160-175 $\geq$ 180	200 and 500  200	1  1	10-15  & 1-15	Only startle response  Deterred	Nestler <i>et al.</i> , 1992
Various species in literature overview									Moulton and Backus, 1955
Red drum	<i>Sciaenops ocellatus</i>								Fuiman <i>et al.</i> , 1999
Atlantic salmon	<i>Salmo salar</i>	Animals in river	10 Hz and 150 Hz	114 dB above hearing threshold	Not specified	Not spec.	10-40 min.	Deterrent effect 10 Hz. No effect 150 Hz,	Enger <i>et al.</i> , 1993. Knudsen <i>et al.</i> , 1994
Silver perch	<i>Bairdiella chrysoura</i>		Bottlenose dolphin whistles					Mating calls reduced by 9 dB	Luczkovich <i>et al.</i> , 2000
Pink snapper	<i>Pagrus auratus</i>	Fish in cages	Air-gun sounds	??	??	??	??	Damage to hair cells of ears	McCauley <i>et al.</i> , 2003
Yellowfin tuna	<i>Thunnus albacares</i>	Dolphin jaw pops, breaches & Tail slaps	200-800 Hz	153-163  141				Calculated detection ranges:380-840 m, 660-1040 m 90-180 m	Finneran <i>et al.</i> , 2000
Sole	<i>Solea solea</i>	Wind noise						Effect on orientation	Lagardère <i>et al.</i> , 1994

Table 2. Continued.

Cod	<i>Gadus morhua</i>	Airguns							Decreased catch with long-lines.	Løkkeborg and Søldal, 1993
Saithe	<i>Pollachius virens</i>	Airguns							Increased catch in trawler nets	Løkkeborg and Søldal, 1993
Cod	<i>Gadus morhua</i>	Seismic shooting							Reduced catch in long lines and trawler	Engås <i>et al.</i> , 1996
Haddock	<i>Melanogrammus aeglefinus</i>	Seismic shooting							Reduced catch in long lines and trawler	Engås <i>et al.</i> , 1996
Rockfish	<i>Sebastes</i> spp.	Air guns	??	??	??	??	??		Either move into the water column or stationary on the bottom	Pearson <i>et al.</i> , 1992
Rockfish	<i>Sebastes</i> spp.	Airguns							Reduced catch in hook-and-line fishery	Skalski <i>et al.</i> , 1992
Sea bass ( <i>Dicentrarchus labrax</i> ), pout ( <i>Trisopterus luscus</i> ), thick lipped grey mullet ( <i>Chelon labrosus</i> ), Atlantic herring ( <i>Clupea harengus</i> ), and cod ( <i>Gadus morhua</i> )		Pingers to reduce bycatch of small cetaceans in gill net								Kastelein <i>et al.</i> , 2005
Overview										Hawkins, 1986
Overview										Popper and Carlson, 1998

Table 3. Mean standard body length of the fish which were subjected to sounds. N = number of individuals, SD = standard deviation. \*Because herring cannot be touched their body length was estimated.

Species	N	Standard body length (cm)		
		Mean	SD	Range
Sea bass	17	22.6	2.4	18-26
Thicklip mullet	11	17	5.3	8-24
Pout	9	20.5	2.7	17.5-24
Cod	5	43.9	1.7	42-46
Eel	10	46.2	6.5	35-57
Pollack	3	24	2	22-26
Horse mackerel	13	3.6	0.8	2.8-4.9
Atlantic herring*	4	27	-	25-30

\*Because herring cannot be touched their body length was estimated.

Table 4. Water temperature during the test periods of the fish species. N = number of measurements, SD = standard deviation.

Fish species	Mean water temperature(°C)	SD (°C)	N	Range (°C)
Sea bass	8.7	1	9	7-10
Thicklip mullet	6.9	0.8	9	6-8
Pout	5.3	1.2	9	3-7
Cod	8.1	0.8	15	7-9
Eel	6.1	0.7	7	5-7
Pollack	10.2	2.9	28	6-16
Horse mackerel	14.4	1.2	15	13-16
Atlantic herring	9.3	0.5	6	9-10

Fig. 1. A schematic view of the study area showing the net enclosure, the location of the three cameras, and the three transducers.

Fig. 2. Background noise level in the tank, expressed in dB re  $1 \mu\text{Pa}^2/\text{Hz}$  - Power Spectrum Density. For comparison the spectrum level curve according Sea state 1 (Wentz, 1962) is also shown.

Fig. 3. The 50 % reaction range curves ( $\pm 8$  dB of average level) for sea bass (100 Hz-700 Hz; school size: 17 fish), the maximum exposure level that could be produced in the tank for tonal signals (causing no reactions by the sea bass), the maximum producible broadband noise exposure level (causing no reactions in the sea bass), and the background noise spectrum level in the tank. Also shown is the ABR audiogram for sea bass (from J. Lovell, 2003; In: Nedwell *et al.*, 2004).

Fig. 4. The 50 % reaction range curves ( $\pm 8$  dB of average level) for thicklip mullet (400 Hz-700 Hz; school size: 11 fish), the maximum exposure level that could be produced in the tank for tonal signals (causing no reactions by the thicklip mullet), the maximum producible broadband noise exposure level (causing no reactions in the thicklip mullet), and the background noise spectrum level in the tank.

Fig. 5. The 50 % reaction range curves ( $\pm 8$  dB of average level) for pout (100 Hz-250 Hz; school size: 9 fish), the maximum exposure level that could be produced in the tank for tonal signals (causing no reactions by the pout), the 50 % reaction threshold exposure level to the broadband noise signal, and the background noise spectrum level in the tank.

Fig. 6. Sound expose levels for cod (school size: 5 fish). The maximum exposure level that could be produced in the tank for tonal signals (causing no reactions by the cod), the maximum producible exposure level of the broadband noise signal (causing no reactions in the cod), and the background noise spectrum level in the tank. Also shown are hearing thresholds of cod obtained by Buerkle (1967) and Chapman & Hawkins (1973).

Fig. 7. Sound expose levels for eel (school size: 10 fish). The maximum exposure level that could be produced in the tank for tonal signals (causing no reactions by the eel), the maximum producible exposure level of the broadband noise signal (causing no reactions in the eel), and the background noise spectrum level in the tank.

Fig. 8. Sound expose levels for pollack (school size: 3 fish). The maximum exposure level that could be produced in the tank for tonal signals (causing no reactions by the pollack), the maximum producible level of the broadband noise signal (causing reactions in the pollack), and the background noise spectrum level in the tank. Also shown is the hearing threshold of pollack obtained by Chapman & Hawkins (1969).

Fig. 9. The 50 % reaction range curves ( $\pm 8$  dB of average level) for horse mackerel (100 Hz-2000 Hz; school size: 13 fish), the maximum exposure level that could be produced in the tank for tonal signals (causing no reactions by the horse mackerel), the 50 % reaction threshold exposure level to the broadband noise signal, and the background noise spectrum level in the tank.

Fig. 10. The 50 % reaction range points ( $\pm 8$  dB of average level) for Atlantic herring (4000 Hz; school size: 4 fish), the maximum exposure level that could be produced in the tank for tonal signals (causing no reactions by the herring), the maximum producible exposure level of the broadband noise signal (causing no reactions in the herring), and the background noise spectrum level in the tank. Also shown is the hearing threshold of herring obtained by Enger (1967).