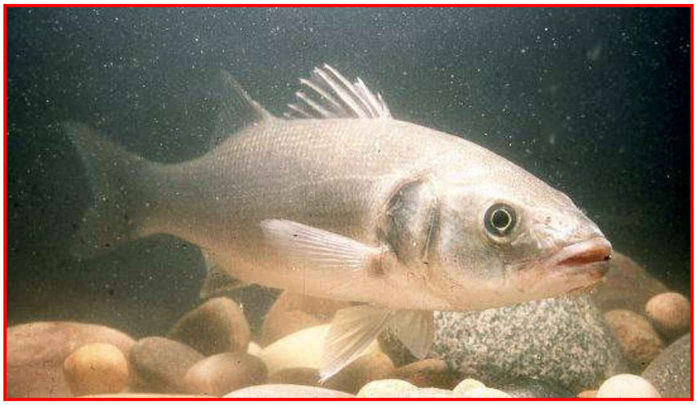
Acoustic dose-behavioral response relationship in sea bass (*Dicentrarchus labrax*) exposed to playbacks of pile driving sounds

SEAMARCO final report 2016-01
(April 2016)



SEAMARCO
SEA MAMMAL RESEARCH COMPANY

Report:
Acoustic dose-behavioral response relationship in sea bass (*Dicentrarchus labrax*)
exposed to playbacks of pile driving sounds

SEAMARCO final report 2016-01 (April 2016)

Authors:
Dr. ir. Ron Kastelein (SEAMARCO)
Aimée Kommeren (SEAMARCO)
Lean Helder-Hoek (SEAMARCO)
Dr. Nancy Jennings (Dotmoth)
Drs. Jessica Schop (SEAMARCO)

Commissioner:
Gemini, Buitengaats C.V.
P.O. number GEM-03-082
Contact: Luuk Folkerts

Contractor:
Dr. ir. R. A. Kastelein
Director & owner
SEAMARCO (Sea Mammal Research Company)
Applied research for marine conservation
Julianalaan 46
3843 CC Harderwijk
The Netherlands
Tel (Office): +31-(0)341-456252
Tel (Mobile): +31- (0)6-46-11-38-72
Fax: +31-(0)341-456732
E-mail: researchteam@zonnet.nl

Acoustic dose-behavioral response relationship in sea bass (*Dicentrarchus labrax*) exposed to playbacks of pile driving sounds

Ronald A. Kastelein[a], Aimée Kommeren, Lean Hoek, Nancy Jennings[b] and Jessica Schop

[a]*Sea Mammal Research Company (SEAMARCO), Julianalaan 46, 3843 CC Harderwijk, The Netherlands*

[b]*Dotmoth, 1 Mendip Villas, Crabtree Lane, Dundry, Bristol BS41 8LN, United Kingdom*

*Corresponding author. Tel.: + 31-341-456252; fax: +31-341-456732.
E-mail address: researchteam@zonnet.nl (R.A. Kastelein).

Abstract

The number of offshore wind turbine parks will increase in the coming decades. So far, most wind turbines have been attached to the ocean floor by means of pile driving. Little is known about the effects of pile driving sounds on marine fish, and information is needed to assess potential environmental impacts. Acoustic dose-behavioral response relationships for pile driving sounds were determined for two size groups of sea bass kept in a large pool. The animals were exposed to played back series of pile driving sounds at seven mean received root-mean-square sound pressure levels (SPLs 130-166 dB re 1 µPa, 6 dB steps; mean received single strike sound exposure level [SEL_{ss}] 122-158 dB re 1µPa^{2}s; mean received single strike particle velocity exposure levels [VEL_{ss}]: 58, 64, 70, 76, 82, 88 and 94 dB re (1nm/s)^2·s.; mean signal duration ~136 ms; strike rate was 2760 strikes/hr; inter-pulse interval of 1.3 s and a duty cycle of ~9.5%). Each session consisted of a 20 min pre-exposure period, a 20 min pile driving playback sound exposure period, and a 20 min post-exposure period. Behavioral responses recorded during each period were classed as startle responses (sudden increases in swimming speed and changes in swimming direction; C-response) which only occurred immediately after the onset of piling sound series, and sustained responses (changes in school cohesion, swimming depth, and relative swimming speed). Clear startle responses were observed; the 50% startle response occurred at a mean SEL_{ss} of 131 dB re 1 µPa^{2}s for the 31 cm fish and 141 dB re 1 µPa^{2}s for the 44 cm fish (add 8 dB to the SEL_{ss} value for SPL dB re 1 µPa; subtract 64 dB from SEL_{ss} value for VEL_{ss} dB re (1nm/s)^2·s.). The sensation level (no. of dB above the 50% hearing threshold) of sounds causing startle responses in 50% of the exposures seems to be 30 - 40 dB in sea bass, depending on the size group. In both size groups, school cohesion varied greatly between the schools in all three periods (pre-exposure, exposure, and post-exposure periods), showing that differences existed in the behavior of the different schools of fish that were tested. However, no sustained behavioral responses to the sounds were observed, suggesting that, under test conditions at least, sea bass of the sizes tested here, after an initial startle response, recover quickly when exposed to regular pile driving sounds at a received SEL_{ss} of at least 158 dB re 1µPa^{2}s (VEL_{ss} 94 dB re (1nm/s)^2·s).

Key words: Acoustics; behavior; marine fish; offshore industry; pile driving; sea bass.

1. Introduction

Many marine organisms rely heavily on acoustics to survive. Fish, for instance, engage with their surroundings through sound, by using species-specific acoustic adaptations for hunting, territorial behavior, mate attraction, spatial orientation, and predator avoidance (Popper *et al*., 2003). Such ecologically important behaviors can be negatively influenced by anthropogenic noise, which often has energy in the low frequencies (Popper and Hastings, 2009), and which is increasing worldwide due to increasing anthropogenic activities (National Research Council, 2003; 2005). However, little is known about the effects of anthropogenic noise on marine fish, and information is needed for realistic environmental impact assessments (Popper et al., 2004; Normandeau Associates, Inc., 2012; Hawkins et al., 2014b).

The number of offshore wind turbine parks in coastal waters will increase worldwide in the coming decades. Most wind turbines are attached to the ocean floor by means of pile driving, which produces sounds of high amplitude and with energy mostly below 1 kHz (Norro *et al*., 2013). Pile driving sounds may negatively affect fish, both behaviorally and physiologically (Popper and Hastings, 2009; Hawkins *et al*., 2014a). Although limited information is available on fish hearing sensitivity (for only about 100 of the 27,000 marine fish species), most audiograms of marine fish species indicate that their greatest sensitivity to sounds falls within the 0.1 – 2 kHz range (Popper *et al*., 2003), overlapping with the spectrum of pile driving sounds.

The effects of pile driving sounds on fish have rarely been studied (Bolle et al., 2012; Halvorsen et al., 2012a,b; Casper et al., 2013a,b; Popper *et al*., 2013; Hawkins *et al*., 2014). The effects of specific sounds on the behavior of marine fish vary greatly depending on the species (Moulton and Backus, 1955; Hawkins, 1986; Myrberg, 1990; Popper and Carlson, 1998; Luczkovich et al., 2000; Kastelein et al., 2007, 2008). Apart from sound parameters and context, the effects of sounds may also depend on the size of the fish, because the size of the swim bladder determines its resonance frequency (Blaxter and Hoss, 1981; Schaefer and Oliver, 1998).

The European sea bass (*Dicentrarchus labrax*) is a fish species which occurs in large numbers throughout the Mediterranean Sea, along the North Sea coasts, and in southwest Norwegian waters (Lart and Green 2011). In its distribution area, many wind farms have been built by using pile driving, and many more will be built in the near future. Offshore pile driving sounds may affect sea bass behavior, since the sea bass hearing sensitivity range (100-1500 Hz; Lovell, 2003) overlaps with the pile driving sound spectrum. Effects of sound on sea bass behavior have been investigated. Kastelein *et al.* (2007) studied the effects of seven commercially available pingers (to reduce harbor porpoise bycatch in fisheries; frequency range: 3-20 kHz): the sea bass decreased their speed in response to one pinger and swam closer to the surface in response to another. Kastelein *et al.* (2008) reported the 50% startle response threshold SPL for sea bass, for tonal signals between 0.1 and 0.7 kHz. Compared to the other animals tested, the sea bass reacted to relatively low sound levels in a relatively wide frequency range (i.e., it was highly responsive to sound). Neo *et al.* (2014) studied the effect of the temporal structure of sounds on behavioral recovery from noise impact in sea bass; intermittent exposure resulted in significantly slower behavioral recovery to pre-exposure levels than continuous exposure. Neo *et al* (2015) found that in impulsive sounds, different pulse repetition rates influenced immediate and delayed behavioral changes in sea bass.

The aim of the present study was to determine the acoustic dose-behavioral response relationship for sea bass exposed to playbacks of a series of pile driving sounds. Both startle responses and sustained responses (changes in school cohesion, swimming depth, and relative swimming speed) were quantified.

2. Materials and methods

2.1. Study animals

European sea bass were selected for testing, based on their economic importance in North Sea fisheries, their availability, their ease of maintenance in captivity, and the temperature range at which they can be kept (the water temperature at the study area was influenced by the environment). The sea bass originated from a commercial hatchery (Ecloserie Marine, Gravelines, France). Two length groups originated from 2 different spawning years. At the time of the study, the mean total body lengths (from the tip of the snout to the tip of the longer lobe of the caudal fin) of the two groups of fish were 31 cm and 44 cm (**Table 1**). Each group was tested in a different year; the small fish in 2013 and the large fish in 2014. The sea bass were tested in schools of 4 fish taken from one of the 2 size groups.

Table 1. Mean standard body length of the sea bass used in the study. N = number of individuals used in the tests, SD = standard deviation. A t-test confirmed that the fish in each group differed significantly in size (T = -17.02, P = 0.000, DF = 48).

Fish group	Standard body length (cm)			
	Mean	SD	N	Range
1 (Small)	30.8	2.3	36	25-35
2 (Large)	44.3	4.0	32	39-53

2.2. Study area

For at least four months before each individual was tested, the fish were kept in their size groups in round white polyester holding tanks 2.2 m in diameter, with a water depth of 1 m. These tanks and their water systems were very quiet (there were no pumps). After a school was tested it was placed in another holding tank to ensure that the fish were not used again.

The experiments were conducted in a large outdoor research pool at the SEAMARCO Research Institute in Wilhelminadorp, The Netherlands. The rectangular pool (7.0 m long, 4.0 m wide; water depth 2.0 m) was made of plywood covered on all sides with fiberglass (**Fig. 1**). It was set into a 1 m deep hole in the ground, resting on a layer of rubber tiles, and the sides below ground level were covered with a layer of 3 cm thick Styrofoam. The pool walls were covered with coconut mats (3 cm long fibers) and the floor was covered with a 20 cm thick layer of sand.

To reduce predation by birds, algal growth, impact of noise from rain, and glistening of the water surface, and to create a more even light pattern, a slanting roof (9 m x 6 m) was built above the pool. To improve the video images, artificial lighting was used during all sessions. The light was switched on at least 10 minutes before a session began.

The water was pumped in continuously from the nearby Oosterschelde (a lagoon of the North Sea), so that all the water in the pool was replaced each day. The salinity was 30 - 33 ‰. To ensure the good water clarity needed to film the fish, the water was circulated via a sand filter. Water temperature was measured daily (range: 10.5-21.5 °C); a previous study (Kastelein et al., 2007) showed that within the temperature range experienced in the present study, the fish reacted to sound independently of the temperature.

To make the environment in the pool 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, and the filtration pump was connected to the pool with flexible rubber hoses.

To ensure that, during test sessions, all fish could be filmed at all times with an underwater camera, the fish being tested were kept in a net enclosure (4.0 m long, 1.75 m wide and 2.5 m high) that was rigged over the width of the pool (**Fig. 1**). The net was made of white nylon (1.5 cm stretched mesh), and kept its shape due to a rectangular PVC frame at the bottom. To increase the contrast between the fish and the sides of the pool for filming, white tarpaulins were placed at the bottom and on three sides of the net enclosure (back and sides). For each session, a school of four individual fish was moved into the net enclosure. The school size of four fish was determined by the availability of the fish, the available space in the net enclosure, and to make the video analysis feasible. In the net enclosure the fish generally showed schooling behavior. A research cabin placed 1 m from the side of the research pool housed the sound generating equipment, monitors, video recording equipment, and sound recording equipment.

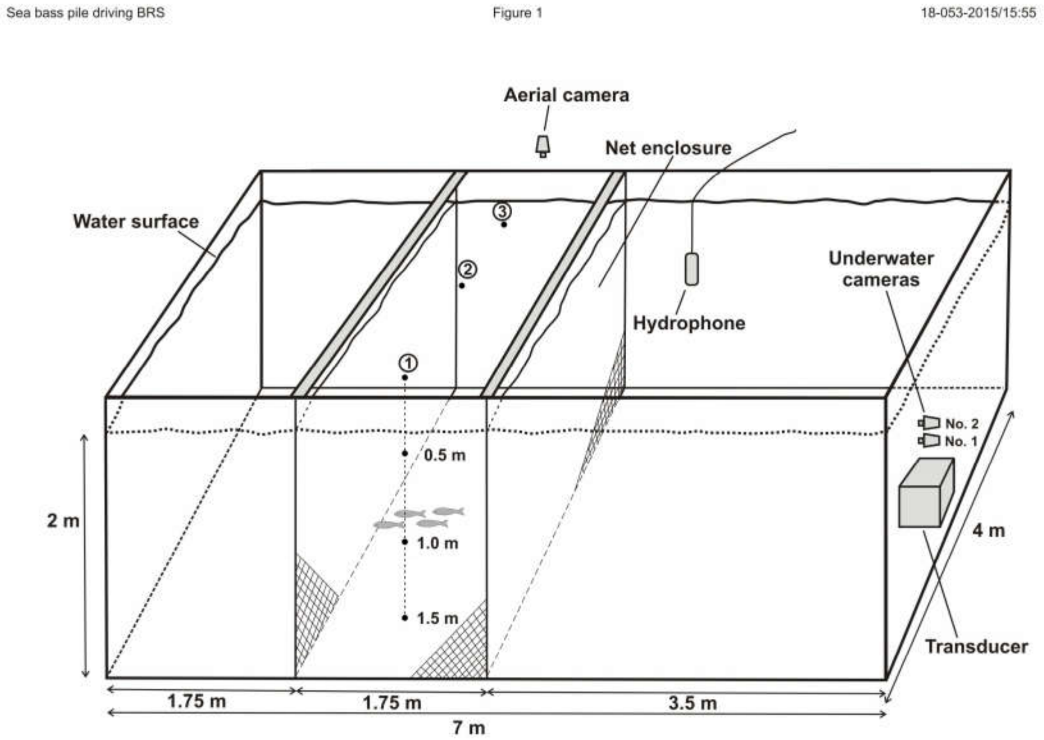


FIG. 1. The research pool in which the acoustic experiments with the sea bass were conducted, indicating the location of the net enclosure, the three cameras, the hydrophone and underwater loudspeaker (transducer) producing the pile driving playback sounds. The fish and pool are drawn approximately to scale. The three sound exposure level (SEL) measurement locations are indicated with numbers ①-③. The SEL was measured at 3 depths per location (0.5, 1.0 and 1.5 m deep).

2.3. Background noise and playback sound measurements

The background noise and played back pile driving noise were measured in the research pool at the beginning and the end of the study period. The sound measurement equipment consisted of three hydrophones [Brüel & Kjaer (B&K) – 8106] with a multichannel high frequency analyzer (B&K PULSE - 3560 D), and a laptop computer with B&K PULSE software (Labshop, version 12.1; sample frequency used: 524288 Hz). Before analysis the recordings were high-pass filtered (cut-off frequency 100 Hz; 3rd order Butterworth filter; 16 dB/octave) to remove low-frequency sounds made by water surface movements. The system was calibrated with a pistonphone (B&K - 4223). The broadband sound pressure level (SPLrootmean-square; dB re 1 μPa) (ANSI, 1994) of pile driving strike sounds was derived from the received 90% energy flux density and the corresponding 90% time duration (t_{90}) (Madsen, 2005).

The received sound pressure of the impulsive sound was analyzed in terms of the $L_{zero\text{-}peak}$ (i.e., 20 times the base-10 logarithm of the maximum absolute value of the instantaneous sound pressure) and the unweighted single strike sound exposure level (SEL_{ss}) in dB re 1 μPa^2s (ANSI, 1986). The SEL was measured at three locations in the horizontal plane in the middle of the net enclosure, and at three depths per location (0.5, 1, and 1.5 m deep; **Fig. 1**).

Because it is not clear whether sea bass react primarily to the sound pressure or to particle motion, not only the SPL was measured in the net enclosure, but also the particle velocity. Sound pressure and particle motion measurements were made using a calibrated 3-D particle motion sensor (Geospectrum Technologies Inc., Model M20) connected to a digital differential oscilloscope (Picoscope, Model 3425 USB). The acoustic data were then analyzed in Matlab (version R2013a) with a bandpass filter applied from 10-3000 Hz, the calibrated range of the vector sensor.

The acoustic metrics zero-to-peak sound pressure level ($SPL_{z\text{-}p}$), zero-to-peak particle velocity level ($PVL_{z\text{-}p}$), single strike sound exposure level (SEL_{ss}), and single strike particle velocity exposure level (VEL_{ss}) were calculated over a period of 1 second during the playback of the pile driving recording using the following the equations:

$$SPL_{z\text{-}p} = 20\log_{10}\left(\frac{\mathrm{Max}(|P(t)|)}{P_{ref}}\right)$$

$$PVL_{z\text{-}p} = 20\log_{10}\left(\frac{\mathrm{Max}(|U(t)|)}{U_{ref}}\right)$$

$$SEL_{ss} = 10\log_{10}\left(\frac{\int_T P(t)^2 dt}{E_{ref}}\right)$$

$$VEL_{ss} = 10\log_{10}\left(\frac{\int_T P(t)^2 dt}{VE_{ref}}\right)$$

P(t) = Instantaneous pressure
U(t) = Instantaneous particle velocity
P_{ref} = Sound pressure reference value
U_{ref} = Particle velocity reference value
VE_{ref} = Particle velocity exposure reference value
E_{ref} = Sound exposure reference value

2.4. Stimulus (playback of pile driving sound)

The fish being tested were subjected to played back series of pile driving sounds. The sounds were recorded at 800 m from a 4.2 m-diameter pile being driven into the sea bed as the foundation for a wind turbine for the Dutch offshore wind farm 'Egmond aan Zee' in the North Sea. The strike rate was 2760 strikes/hr, the inter-pulse interval 1.3 s and the duty cycle ~9.5%. A WAV file was made of series of consecutive pile driving strike sounds. The original recordings were sampled at 65 kHz and high-pass filtered at a cut-off frequency of 50 Hz. For the generation of the WAV files used in the study, signals were resampled to 88.2 kHz.

A random section of five strikes from the digitized original recording of series of pile driving sounds (the WAV file) was played back repeatedly by a laptop computer (Acer Aspire

ZRI) with a program written in LabVIEW, to an external data acquisition card (National Instruments - USB 6361), the output of which could be controlled in 1 dB steps with the LabVIEW program. The output of the card went through a custom-built buffer and filter, to a power amplifier (Crown - 5000VZ), which drove the transducer (Lubell - LL1424HP) through an isolation transformer (Lubell - AC1424HP). The transducer was placed on the bottom at the south-eastern end of the pool at 2 m depth (**Fig. 1**).

Sound pressure measurements

The linearity of the system emitting the pile driving sounds was checked during each calibration, and was found to be consistent to 1 dB within a 20 dB range.

The maximum SEL of the pile driving playback sounds produced during the study was at the maximum level of the sound emitting system, without causing distortion of the signal. This resulted in a maximum mean single strike SEL (SEL_{ss}) of 158 dB re 1 $\mu Pa^2 s$, which is a mean SPL of 166 dB re 1 μPa (based on 9 measurements in the middle of the net enclosure; 3 locations, 3 depths each; **Fig. 1**). The mean duration of the playback, defined as the time interval between the arrival of 5% and 95% of the total energy (t_{90}; Madsen, 2005), was ~136 ms (range 129-143 ms), depending on the SPL (due to reverberations). Most of the energy was in the 1/3 octave band centered at 630 Hz (**Fig. 2**). The waveforms of the original recording at sea and of the recording of the playback sound in the research pool are shown in **Figure 3**. The SEL in the net enclosure varied little due to reverberations in the pool; it varied by at most 2 dB between the 3 locations per depth and at most 3 dB between the three depths per location.

During a three-week pilot study with two schools of fish (that were not used during the main experiment), the signal SELs for the main study were determined by decreasing the SELs from the maximum that could be produced without deformation of the signal, until no behavioral response was observed in the fish. The range was from SEL_{ss} 122 dB re 1$\mu Pa^2 s$ (no response) to SEL_{ss} 158 dB re 1 $\mu Pa^2 s$ (maximum producible level without distortion of the signal). The range found was divided into 6 dB steps, resulting in seven SELs to be tested (mean SEL_{ss}: 122, 128, 134, 140, 146, 152 and 158 dB re 1 $\mu Pa^2 s$; mean SPL: 130, 136, 142, 148, 154, 160, 166 dB re 1 μPa).

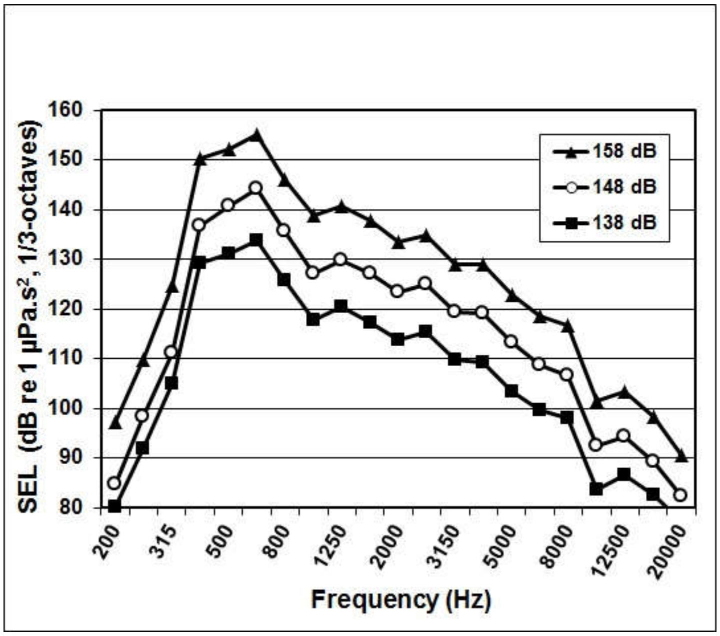


FIG. 2. The 1/3-octave band SEL spectrum of a single played back pile driving sound measured in the net enclosure (location 2 at 1 m depth; see **Figure 1**) at 3 source levels (10 dB steps). The SEL_{ss} of 158 dB re 1 µPa^{2}s shown was the highest level produced in the study. The other 6 SELs the fish were exposed to in the study were in steps of 6 dB lower (the 138 and 148 dB levels shown here were not used in the study, but indicate that the spectrum remained the same at different source levels). The 1/3-octave band centered at 630 Hz contained the most energy (the resonance frequency of the transducer was at 600 Hz). Note that the shape of the spectrum remained the same for all source levels shown.

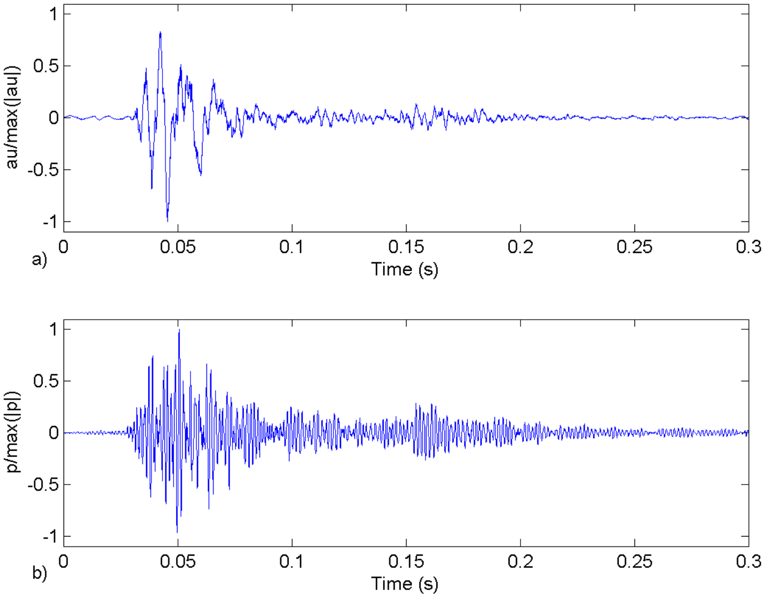


FIG. 3. Waveforms of pile driving strike sounds: a) the original recording, made at 800 m from the pile driving site, derived from the WAV files (au = arbitrary unit) ; b) a played back pile driving sound in the research pool. The amplitude of the sound pressure is scaled to the maximum absolute value of the instantaneous sound pressure. Note the clear reverberations in the sound recording in the pool.

Before a session began, the sound generating equipment was checked by playing a WAV file with a 1 kHz continuous wave. The output of the amplifier was measured with a voltmeter (GWInstek GDM8251A) and an oscilloscope (Voltcraft 632FG). If the output was the same as during the calibrations, a test session could begin.

During test sessions the played back pile driving sounds and background noise were checked for consistency with a custom built hydrophone (10 Hz-120 kHz), a charge amplifier (CCAMS1000-1) and an amplified loudspeaker. The spectra of the sounds were checked for consistency with a spectrum analyzer (Velleman PCSU-1000) on a laptop (Acer Aspire NAV50).

The results of the recordings at 1 m depth at locations 1 and 3 with the M20 sensor (which contains a 3-D particle motion sensor and a hydrophone) are shown in Table 2. The SEL_{ss} measurements with the M20 and the B&K equipment varied 0 and 2 dB depending on the measurement position. During the study the sea bass were exposed to the following six single strike particle velocity exposure levels (VEL_{ss}): 58, 64, 70, 76, 82, 88 and 94 dB re $(1nm/s)^2 \cdot s$.

Table 2. The zero to peak sound pressure level (SPL_{z-p}), zero-to-peak particle velocity level (PVL_{z-p}), single strike sound exposure level (SEL_{ss}), and single strike particle velocity exposure level (VEL_{ss}) were calculated over a period of 1 s recorded with the M20 sensor at locations 1 and 3 (**Fig. 1**). Recording depth: 1 m. Relative attenuation level -19 dB to avoid clipping of the M20 sensor (corresponding to SEL_{ss} 140 dB re $1\mu Pa^2 \cdot s$ measured with the B&K equipment).

Parameter	Unit	Position 1	Position 3
SPL_{z-p}	dB ref 1µPa	155	154
PVL_{z-p}	dB ref 1nm/s	95	95
SEL_{ss}	dB re $1\mu Pa^2 \cdot s$	142	140
VEL_{ss}	dB re $(1nm/s)^2 \cdot s$	76	75

2.5. Observation equipment

The behavior of the fish was recorded from one side with underwater video camera no. 1 (GOPRO®, HERO3). The camera was mounted in the middle of the south-eastern side of the research pool at a depth of 1 m (**Fig. 1**); its wide-angle lens made the entire net enclosure visible in the video image. The images from this camera were used for the analysis of behavior. The camera also recorded the pile driving playback sounds. Camera no. 1 was mounted on a PVC tube, immediately below another underwater video camera (no. 2, SC 2000), the image from which was used for monitoring during sessions and could be seen by the operator on a laptop screen (ACER, KAV60). By viewing the image from camera no. 2 while adjusting the position of the PVC tube, operators could optimize the image from camera no. 1 so that the net enclosure was fully visible.

An aerial camera (SC 2000) filmed the fish from above. The images from this camera were made visible to the researcher on a laptop computer (Acer model KAV60) in the research cabin, and served to monitor the fish during the sessions and as a backup.

Via a microphone (Zetagi), the operator added the date, session number, and fish size to the video recordings. The outputs of the charge amplifier and the microphone were fed into the EZ grabbers, so that video and audio were synchronized. Thus, the behavior of the fish at the exact times of stimulus presentation could be analyzed later.

2.6. Methodology

The sea bass were tested in schools of four fish of similar size. The fish of each school were randomly selected from each size group in the holding tanks. In the holding tanks, the animals were fed *ad lib.* on pieces of raw fish (food was given until the animals stopped eating) twice a week. The amount eaten depended on the water temperature, as water temperature determines the body temperature and thus the metabolic rate of fish.

A school of four fish was removed from the holding tank and placed in the net enclosure in the research pool at least two days before the first session was conducted, which allowed the fish to acclimatize to the enclosure in the research pen (no test sounds were produced in that acclimation period). The transducer was placed in the pool at the beginning of each working day and remained there until the end of the day. Camera no. 1 was mounted two minutes prior to each session. As the pump in the pool was quiet, it was left on during the experiments, but the valve for sea water supply was closed so that no extra water entered the pool and spilled over the skimmer, and no skimming sound occurred.

A session consisted of a 20 min pre-exposure period, followed by a 20 min test period (exposure to played back pile driving sound), and a 20 min post-exposure period. Within each 20 min exposure session, the animals were exposed to a playback consisting of 920 pile driving strike sounds. One or two sessions were conducted daily between 08.30 and 16.00 hrs with an interval of at least three hours. Sessions were conducted 5 days per week. Each school of fish was in the research pool for 14 days (2 days acclimation over the weekend, and 10 test days during working days in the following 2 weeks, plus the intervening weekend. This resulted in 14 sessions with each school in 10 working days).

In each session, the fish were exposed to sounds at one SPL. The 7 SPLs were tested twice per school, but some of the recordings (5%) were not good enough for analysis (too dark, camera image not covering the entire net area, camera stopped before the entire session was conducted). For each school, the sessions with each of the 7 SPLs were conducted in random order during the 10 working days. The study was conducted between June and November 2013 (the pilot study, plus tests on 9 schools, each with 4 small fish of a mean length of 31 cm), and between July and August 2014 (tests on 8 schools, each with 4 large fish of a mean length of 44 cm). Each of the 68 fish in the study spent only one 14-day period in the research pool (**Table 1**).

Great care was taken to make the test environment as quiet as possible. Only the researcher involved in the test was allowed within 5 m of the research pool during test sessions. During test sessions the background noise in the pool was very low (i.e., below the sound of Sea State 0, thus not influencing the results; see Kastelein *et al*., 2007).

All recordings were coded for date and session number, so that analysis could be conducted partially blind. The analysts (who did not record the sessions) knew what size of fish was being tested and whether the period was pre-exposure, exposure or post-exposure, but was not aware of the sound level.

Table 1. Each school of 4 sea bass was in the net enclosure for 14 days: two weekend days of acclimation (Accl.), followed by 5 test days, followed by 2 weekend days without sound exposure, followed by 5 test days. In the 10 test days, the fish were exposed twice to each of the 7 SPL (on some random days 2 sessions were conducted).

Day in enclosure	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Week day	Sat	Sun	Mon	Teu	Wed	Thur	Fri	Sat	Sun	Mon	Teu	Wed	Thur	Fri
Activity	Accl.	Accl.	Test	Test	Test	Test	Test			Test	Test	Test	Test	Test
No. of sessions/day (example)			1	2	1	2	1			1	2	2	1	1
SPL (example)			1	3&7	4	2&5	6			2	1&5	4&6	3	7

Startle response

A startle response (the tail-flip or Mauthner reflex; Eaton et al., 1977) to a stimulus was characterized by a sudden change in swimming speed, swimming direction, and body posture, and only (depending on the SEL of the pile driving sound playback) occurred just after the onset of the acoustic signal presentation (Blaxter et al., 1981). The first response had to occur in the first 2 seconds of the sound exposure. If at least one of the fish in the school reacted to the stimulus, the session was classified as having a startle response.

The video images were analyzed independently by two analysts who were unaware of the SPL of the played back pile driving sound. There was no reason for the analysts to be biased, as we were not expecting or predicting any particular response (or lack of response) to the pile driving sound. The startle responses of the fish were in fact so clear that no disagreement between the ratings of the two analysts occurred throughout the study, and startle responses were not observed outside the pile driving sound playback exposure periods.

Sustained responses

Recordings from underwater camera no. 1 were used to quantify sustained responses as changes in school cohesion, swimming depth, and relative swimming speed. During the pre-exposure, test, and post-exposure period of each session, an observation of school cohesion, swimming depth, and relative swimming speed was made every 2 min, resulting in 10 measurements per 20-minute period. The first pre-exposure measurement was 19 min before the start of sound exposure (T 0). The first test measurement was 1 min after the start of exposure, and the first post-exposure measurement was 1 min after exposure stopped. The mean of the 10 measurements for each period was used for analysis.

In order to quantify school cohesion, the distance between the center of each fish making up each pair of fish in the school (1-2, 1-3, 1-4, 2-3, 2-4, 3-4) was measured in cm from the computer screen (0.5 cm accuracy). Per recording moment, school cohesion was determined as the average distance between the 4 sea bass (the mean of 6 measurements). A large distance meant that the fish were far apart or spread out within the research pool (weak cohesion); a small distance meant that the fish were schooling close together (tight cohesion).

Swimming depth within the net enclosure was quantified by allocating each fish in the school to one of four depths within the water column (depth 4 represented the bottom quarter of the research pool, depth 1 represented the top quarter of the pool), and calculating the school mean. A grid was superimposed over the computer screen to determine at which depth the sea bass were. The center of each fish was used to determine its position; when the fish's center was at the boundary between two depths, the depth in the direction in which the fish was swimming was recorded.

At each scoring moment during test and post-exposure periods, the swimming speed relative to the general impression of speed during pre-exposure periods (a subjective measure) was recorded per animal as +1 (faster), 0 (similar), or -1 (slower).

2.7. Analysis

Startle response data (response or no response for at least one fish, per session) were submitted to probit analysis with the stimulus 'level' and the factor 'fish group' (small or large).

Sustained response data (mean school cohesion, mean swimming depth, and mean relative swimming speed) were first submitted to correlation analysis in order to investigate relationships between the three variables and to check for autocorrelation. Autocorrelation did exist, and school cohesion was shown by correlation analysis to be predictive of the other variables (see Results), so this variable only was chosen for further analysis.

For the analysis of school cohesion, a separate repeated-measures ANOVA was carried out for each size of fish. Values for school cohesion were submitted to a model with the random factor 'school' (subject) and the within-subjects fixed factors 'level', and 'period' (pre-exposure, test and post-exposure). The interaction term ('level' x 'period') was included in both initial models, but was not significant, so it was excluded from both final models.

All analysis was carried out with $\alpha = 0{\cdot}05$, by using probit analysis and the General Linear Model procedure, in Minitab 17 statistical software (www.minitab.com); data conformed to the assumptions of the tests used.

3. Results

Startle response

The number of schools which showed a startle response at each of the 7 mean received SEL_{ss} is shown in **Table 2**.

Probit analysis showed that louder sounds were more likely to elicit a startle response than quieter sounds, and that small fish responded to quieter sounds than large fish: both the stimulus level (regression coefficient = 0.07, SE = 0.009, Z = 7.51, P = 0.000) and the size of fish (regression coefficient = 0.66, SE = 0.191, Z = 3.47, P = 0.001) had significant effects on the probability of fish showing startle responses (**Fig. 4**). There was no significant difference in the pattern of response for each of the two size groups (test for equal slopes: $\chi^2 = 0.234$; DF = 1, P = 0.629). A Pearson test showed that goodness-of-fit for the model was adequate ($\chi^2 = 13.6$; DF = 11, P = 0.258).

For small fish, the 50% startle response occurred at a mean SEL_{ss} of 131.2 dB re 1 μPa^2s (SE = 2.2, 95% CI = 126.9 - 135.4). For large fish, the 50% startle response occurred at a mean SEL_{ss} of 141.1 dB re 1 μPa^2s (SE = 2.0, 95% CI = 137.2 - 145.0).

Table 2. The number of schools which showed a startle response at each of the 7 mean received SEL$_{ss}$.

	Big fish						Small fish					
Mean SEL$_{ss}$	No response		Startle response		Total		No response		Startle response		Total	
dB	n	%	n	%	n	%						
122	14	88	2	12	16	100	10	83	2	17	12	100
128	14	88	2	12	16	100	9	50	9	50	18	100
134	11	73	4	27	15	100	8	44	10	56	18	100
140	7	44	9	56	16	100	4	22	14	78	18	100
146	5	31	11	69	16	100	5	28	13	72	18	100
152	1	7	14	93	15	100	1	6	17	94	18	100
158	5	33	10	67	15	100	0	0	18	100	18	100

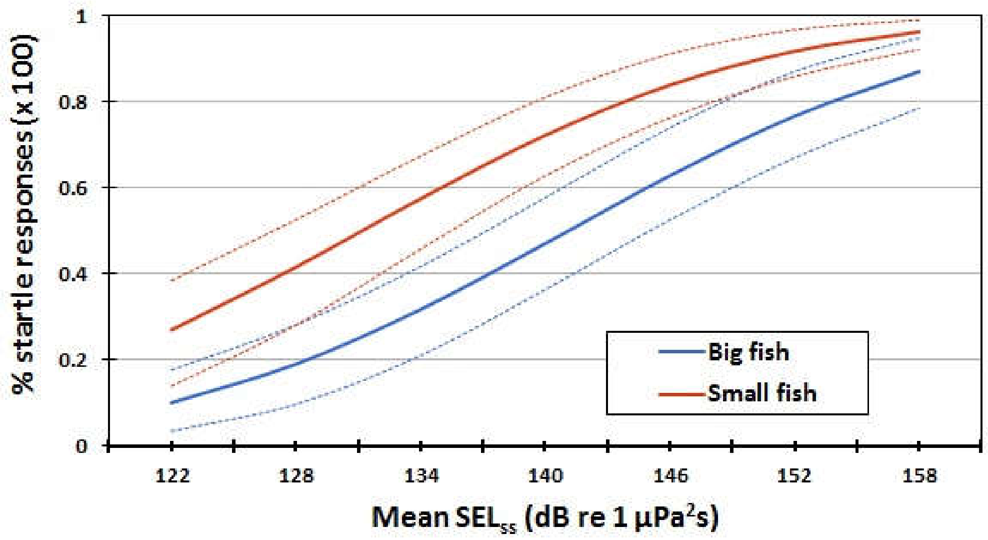


FIG. 4. Cumulative reaction plot, derived from probit analysis, showing modelled % startle responses of large fish (solid blue line) and small fish (solid red line) to pile driving sounds of increasing SEL$_{ss}$ in dB re 1 µPa^{2}s (the acoustic dose). Also shown are the 95% CIs (upper and lower limits, dashed lines).

Sustained response: school cohesion

Correlation analysis, applied in order to investigate relationships between the three sustained response variables and to check for autocorrelation, showed that significant relationships existed for both sizes of fish between school cohesion and both swimming depth and relative swimming speed. Swimming depth and relative swimming speed were not correlated with one another. When swimming higher in the water column (less deep), the fish tended to school more closely together (tighter cohesion). When swimming faster, the fish also tended to school more closely together. Since school cohesion was predictive of both the other sustained response variables, and was the only variable derived from continuous data and conforming to a normal distribution, further detailed analysis of the sustained response was carried out only with this variable.

In the large fish (mean size 44 cm), the repeated-measures ANOVA on school cohesion with the random factor 'school' and fixed factors 'level' and 'period' (pre-exposure, test, and post-exposure) revealed that school cohesion was not significantly affected by 'level' (DF = 6, adjusted MS = 2.68, F = 1.71, P = 0.117) or by 'period' (DF = 2, adjusted MS = 2.23, F = 1.04, P = 0.355). The random factor 'school' had a significant effect on school cohesion (DF = 7, adjusted MS = 38.7, F = 18.0, P = 0.000), showing that individual differences existed in the behavior of the different schools of fish that were tested. Most of the variation in school cohesion could be attributed to these individual differences. Even in a reduced repeated-measures ANOVA including only the highest level (SEL_{ss} 158 dB re 1 $\mu Pa^2 s$; not including the factor 'level'), school cohesion was not affected by 'period' (DF = 2, adjusted MS = 2.06, F = 1.94, P = 0.160).

In the small fish (mean size 31 cm), the repeated-measures ANOVA on school cohesion with the random factor 'school' and the fixed factors 'level' and 'period' (pre-exposure, test and post-exposure) revealed that school cohesion was significantly affected by 'level' (DF = 6, adjusted MS = 11.5, F = 4.80, P = 0.000), but not by 'period' (DF = 2, adjusted MS = 6.18, F = 2.57, P = 0.078). Post-hoc Tukey pairwise comparisons of the levels revealed significant differences in school cohesion only between SEL_{ss} 128 dB re 1 $\mu Pa^2 s$ (weaker school cohesion; i.e., larger mean distance between fish in a school), and 140 dB re 1 $\mu Pa^2 s$ (tighter school cohesion; i.e., smaller mean distance between fish in a school). The random factor 'school' also had a significant effect on school cohesion (DF = 8, adjusted MS = 40.8, F = 17.0, P = 0.000), showing that individual differences existed in the behavior of the different schools of fish. Most of the variation in school cohesion could be attributed to these individual differences. Even in a reduced repeated-measures ANOVA including only the highest level (SEL_{ss} 158 dB re 1 $\mu Pa^2 s$; not including the factor 'level'), school cohesion was not affected by 'period' (DF = 2, adjusted MS = 5.89, F = 3.13, P = 0.056). Cohesion did become tighter during the test period, but not significantly.

In both small and large fish, initial ANOVAS (not shown; non-significant interaction terms removed from the final models shown above) revealed that the interaction term between 'level' and 'period' was not significant, showing that fish responded similarly at all sound levels and in all periods: the pattern of response was consistent.

Overall, analysis showed that there is no evidence, even at the highest sound level, for any consistent sustained response to sound exposure by the study animals.

4. Discussion and conclusions

4.1. Evaluation

In quantifying startle responses, we judged that the two analysts used consistent criteria, because their classifications of the startle behaviors were identical (the startle responses were very obvious). Startle responses were not observed outside the pile driving sound playback exposure periods; this was judged from. casual observations made during equipment set-up and between sessions, when the underwater recording systems were switched on.

The size of their enclosure influences the general swimming behavior of many fish species. Before the fish were put in the net enclosure in the large research pool, they were kept in much smaller circular holding tanks, in which they swam very slowly or not at all; instead they 'hovered' most of the time. In the net enclosure in the large research pool, the fish were much more active; they behaved in the same way as fish in a previous study that had the entire pool available to them (Kastelein et al., 2007). So, although the research pool was far from a natural environment, it was a much better study area than the smaller tanks used in many studies on responses of marine fish to sound.

The study fish had been housed in tanks all their lives. However, the facility where the animals came from had water filtration systems that were relatively quiet, so the study animals had probably not been exposed to higher sound levels than wild conspecifics. The site for the SEAMARCO Research Institute was selected because of its remote location and quiet environment, the research pool was designed specifically for acoustic research, and the area around the pool was strictly controlled (nobody was present within 5 m, except the researcher who sat quietly in the research cabin), so there was little background noise.

The responses of the fish in the present study were probably dependent on the context in which the sounds were produced, and may not have been representative of sea bass in the wild. However, even in the wild, animals behave differently depending on parameters such as location, time of day, water temperature, their history, physiological state, age, body size, and school size. Therefore, the present study gives a rough indication of SPLs to which sea bass at sea may show a startle response , and an SPL below which probably no startle response will occur. The 50% startle response occurred at a mean SEL_{ss} of 131 dB re 1 μPa^2s for the 31 cm fish and 141 dB re 1 μPa^2s for the 44 cm fish (add 8 dB to the SEL_{ss} value for SPL dB re 1 µPa; subtract 64 dB from SEL_{ss} value for VEL_{ss} dB re $(1nm/s)^2 \cdot s$.).

The school size probably had an influence on the responses of the fish in the school. One responsive fish may trigger a reaction in the other fish of a school, and conversely, fish may feel more secure in a school if the other fish are less responsive to sound. Thus, bigger schools are more likely (just by chance) to contain at least one responsive individual.

4.2. The startle response threshold SEL_{ss}

The 50% startle response occurred at a mean SEL_{ss} of 131.2 dB re 1 μPa^2s for small fish and 141.1 dB re 1 μPa^2s for large fish. Thus a 10 dB difference existed in startle response threshold SPL between the two fish sizes: the small fish were acoustically more sensitive than the large fish. There are at least three possible explanations for this difference:

1) The resonance frequency of the swim bladder of the small fish was more in tune with the frequency in the spectrum with most energy (600 Hz), and thus the small fish experienced the pile driving sounds differently (as being louder or causing a different sensation) than the large fish. Among several parameters, the effect of sound depends on the size of the fish, because the size of the swim bladder determines its resonance frequency (Schaefer and Oliver, 1998).

2) A startle response to sound resembles an anti-predator response (escape behavior). Smaller fish have more potential predators than larger fish. Therefore, they may have to be more vigilant to avoid predation.

3) The larger fish were approximately one year older than the small fish and were therefore more experienced with life in general, and had spent more time in the holding tanks. Their experiences were different, but several parameters were similar for each group: both originated from the same fish farm, each group was housed at the SEAMARCO Research Institute in the same holding tanks and test tank, and at the same water temperature range. Also the equipment set-up and methodology was exactly the same for each group.

Blaxter and Hoss (1981) also documented a difference in startle response sensitivity to 70-200 Hz signals between herring (*Clupea harengus*) of different sizes (test range 2.8-17 cm); the most sensitive fish were in the length range of 8-11 cm (i.e., in the middle of the length range they tested).

Hawkins et al (2014) recorded the behavior of wild schools of sprat (*Sprattus sprattus*) and mackerel (*Scomber scombrus*) in response to a short sequence of impulsive sound playbacks, simulating the strikes from a pile driver, in a quiet coastal location at different sound pressure levels. The incidence of behavioral responses increased with increasing sound level. Sprat schools were more likely to disperse and mackerel schools were more likely to change depth in response to sounds. The sound pressure levels to which the fish schools responded in 50% of presentations were 163.2 and 163.3 dB re 1 µPa peak-to-peak, and the SEL_{ss} were 135.0 and 142.0 dB re 1 μPa^2 s, for sprat and mackerel, respectively, estimated from dose response curves. These 50% behavioral response threshold levels are very similar to the threshold SEL_{ss} found in the present study for startle responses in sea bass. However, the 50% startle response SEL_{ss} are likely to be species-specific, as was pointed out by Nedwell et al. (2006).

4.3. Recovery

Though they did show a startle response, the sea bass in the present study showed no sustained behavioral response to exposure to the pile driving sounds. Even at the highest mean received SEL_{ss} of 158 dB re $1\mu Pa^2 s$, there was no statistical difference in mean school cohesion, which was predictive of the other sustained response behavioral parameters, during the pre-exposure, exposure, and post exposure periods. This suggests that the animals recovered quickly after the initial startle response.

A decrease in behavioral response over time (recovery) does not necessarily indicate that habituation (learning to stop responding to a stimulus which is no longer biologically relevant; Rankin et al., 2009) has taken place. Apparent recovery may occur because:

1) animals hear selectively, filtering out repeated or irrelevant sound signals in the background, in the same way that humans filter out the ticking of a clock (Rankin et al. 2009);
2) the sensitivity of the hearing organs is reduced by loud exposures, leading to temporary hearing threshold shift (TTS); or
3) animals suffer motor fatigue, and become unresponsive due to exhaustion (Domjan 2010).

It is important to determine the mechanism of recovery, since the different mechanisms have different ecological implications. In the present study, apparent recovery is clearly not due to motor fatigue, as the animals' swimming speed only increased for less than 30 sec after the pile driving playback sound started. The inter-pulse interval and signal durations were regular in the present study, so the fish may have become accustomed to the sound and been able to filter it out. Neo et al. (2014) showed that behavioral recovery in sea bass was faster after exposure to regular sounds than to irregular sounds. This phenomenon was also observed in rats (Davis 1970). By exposing sea bass in the same facility to a tone after their exposure to impulsive sounds at similar levels to those used in the present study, Neo et al. (2015) showed that the reduced behavioral response was due to habituation (as the fish reacted to the tone to the same degree as they reacted to the start of the impulsive sound).

After the sound was switched on, depending on the received SEL_{ss}, the fish in the present study usually swam faster, and in more tightly cohesive schools. However, these changes only occurred for a very short time (less than 2 min) and were not apparent (relative to the pre-exposure behaviors) when averaged over the 10 behavioral recordings of the 20 min periods. These behavioral changes constitute the startle response. Neo et al. (2014) also showed that sea bass dove deeper and swam in more compact schools (tighter school cohesion) after sound was switched on, but in their study the behavior of the sea bass returned to pre-exposure levels more gradually (possibly because the sounds they used differed from the ones used in the present study).

Fewtrell and McCauley (2012) observed alarm responses in a teleost fish species in response to impulsive sounds. White trevally (*Pseudocaranx dentexa*) of undetermined length swam faster, in tighter groups and towards the bottom of the cage during exposure to air gun sounds (estimated received SEL_{ss}: 147- 162 dB re 1 µPa^2.s; rate: 1 signal per 10 s). The change in these three behaviors increased as noise levels increased. The fish returned to their pre-noise exposure position in the water column within 31 min after the final air gun signal of the trial. These observations are, in part, similar to those of the present study. Researchers in both studies used impulsive sounds, but with different inter-pulse intervals.

4.4. Sound exposure guidelines for sea bass

During recent years, underwater sound has been of increasing interest to governments (e.g. National Research Council, 2003), as they have to set standards, for example, for acceptable sound levels for marine animals.

Popper et al. (2014) proposed guidelines for safe levels of pile driving sound. However, for fish species with swim bladders not involved in hearing, such as the sea bass, guidelines are given only to avoid mortal injury (cumulative Sound Exposure Level (SEL_{cum}) 210 dB re 1 µPa^2 or > 207 dB peak), recoverable injury (SEL_{cum} 203 dB re 1 µPa^2 or > 207 dB peak), and TTS (> SEL_{cum} 186 dB re 1 µPa^2). These values are obviously much higher than those found in the present study, which was focused on behavioral responses. For behavioral response criteria, SEL_{ss}, SPL, or VEL_{ss} are probably better units than SEL_{cum}; SEL_{cum} is more suitable for injury and TTS.

For convenience, and because of a lack of knowledge, fixed levels above the basic hearing threshold of animals (weighted levels) have often been used as criteria for acceptable sound levels (National Research Council, 2005; Nedwell et al., 2007; Southall et al., 2007). Therefore, we compared the startle response threshold levels we found in the present study to hearing threshold levels of sea bass, to evaluate the relationship between the hearing threshold level of a sound and the response threshold level to it.

The hearing sensitivity of sea bass has been tested physiologically (auditory brainstem response method; Lovell, 2003). The background noise level in the research pool used in the present study was sufficiently low not to mask the high-energy pile driving playback sounds.

In the sea bass, the 50% startle response threshold SPLs for tonal signals were 0-30 dB (depending on the frequency; Kastelein et al., 2008) above the hearing threshold SPLs for the test frequencies (Lovell, 2003). For 600 Hz (the peak frequency of the pile driving sound spectrum in the present study; **Fig. 2**), the hearing threshold of the sea bass is ~110 dB re 1 µPa (Lovell, 2003). For 600 Hz tonal signals, the 50% startle response SPL was between 140 and 160 dB re 1 µPa (Kastelein et al., 2008). In the present study (using impulsive sounds), the SPLs which caused startle responses in 50% of the exposures were 139 dB re 1 µPa for the small fish and 149 dB re 1 µPa for the large fish. These values are similar to those for 600 Hz tonal signals. Thus the sensation level (no. of dB above the 50% detection threshold) causing startle responses in 50% of the exposures seems to be 30 - 40 dB in sea bass, depending on the size group.

Nedwell et al. (2007) proposed a set of guidelines for behavioral impact assessment for fish and marine mammals, utilizing dBht (the number of dB above a species' hearing threshold; i.e. the sensation level). They suggested that the following sensation levels elicit particular responses: 0–50 dB elicits a mild response in a minority of individuals, probably not sustained; 50–90 dB elicits a stronger response in the majority of individuals, but habituation may limit the effect; 90 dB and above elicits a strong avoidance response in virtually all individuals; above 110 dB is the tolerance limit of sound; unbearably loud. The 30 - 40 dB sensation level found in the present study for sea bass fits into the 0 – 50 dB category proposed by Nedwell et al. (2007), as the responses of the fish were not sustained.

Acknowledgements

We thank Rob Triesscheijn for making some of the figures and Arie Smink for the design, construction, and maintenance of the electronic equipment. We thank Merlijn de Graaf for her advice on analyzing the video recordings, Naomi Claeys and Celine van Putten for help with the video recordings and Jennifer Covi for help at the start of the analysis.

We thank Bert Meijering (Topsy Baits) for providing space for the SEAMARCO Research Institute. The sound measurements were conducted by Erwin Jansen (TNO, The Hague). The particle velocity measurements and analysis were conducted by Errol Neo and James Campbell (Leiden University, Netherlands).

We thank Errol Neo (University of Leiden, Netherlands), Luuk Folkerts (Gemini wind park, Netherlands), Inger Inger van den Bosch (RWS, Netherlands) and Aylin Erkman (RWS, Netherlands) for their comments on this manuscript. This project complied with the Dutch standards for animal experiments, and was funded by Gemini offshore wind farm (P.O. number GEM-03-082).

References

ANSI, American National Standard S1.1-1994 (R2004). (American National Standards Institute, New York).

ANSI , American National Standard S12.7-1986 (R2006)., 1986. “Methods for measurement of impulse noise” (American National Standards Institute, New York).

Blaxter, J.H.S., and Hoss, D.E., 1981. Startle response in herring: the effect of sound stimulus frequency, size of fish, and selective interference with the acoustico-lateralis system. Journal of the Marine Biology Association U.K. 61, 871-879.

Blaxter, J.H.S., Gray, J.A.B., Denton, E.J., 1981. Sound and startle response in herring shoals. Journal of the Marine Biology Association U.K. 61, 851-869.

Bolle, L. J., de Jong, C. A., Bierman, S. M., van Beek, P. J., van Keeken, O. A., Wessels, P. W., van Damme, C. J., Winter, H. V., de Haan, D., and Dekeling, R. P. (2012). Common sole larvae survive high levels of pile-driving sound in controlled exposure experiments. PLoS ONE 7, e33052. doi:10.1371/ journal.pone.0033052

Casper, B. M., Popper, A. N., Matthews, F., Carlson, T. J., and Halvorsen, M. B. (2012). Recovery of barotrauma injuries in Chinook salmon,*Oncorhynchus tshawytscha* from exposure to pile driving sound. PLoS ONE, 7(6): e39593. doi:10.1371/journal.pone.0039593.

Casper, B. M., Smith, M. E., Halvorsen, M. B., Sun, H., Carlson, T. J., and Popper, A. N. (2013a). Effects of exposure to pile driving sounds on fish inner ear tissues. Comparative Biochemistry and Physiology A, 166:352-360.

Casper, B. M. Halvorsen, M. B., Mathews, F., Carlson, T. J., and Popper, A. N. (2013b). Recovery of barotrauma injuries resulting from exposure to pile driving sounds in two sizes of hybrid striped bass. PLoS ONE, 8(9): e73844. doi:10.1371/journal.pone.0073844

Davis M (1970) Effects of interstimulus interval length and variability on startle-response habituation in the rat. J Comp Physiol Psychol 72:177–192

Domjan M (2010) The Principles of Learning and Behaviour, 6th edn. Wadsworth, Cengage Learning, Belmont, CA

Eaton RC, Bombardieri RA, Meyer DL (1977) The Mauthner-initiated startle response in teleost fish. J Exp Biol 66:65–81

Fewtrell, J.L., McCauley RD (2012) Impact of air gun noise on the behaviour of marine fish and squid. Mar Pollut Bull 64:984–93

Halvorsen, M. B., Casper, B. M, Woodley, C. M., Carlson, T. J., and Popper, A. N. (2012a). Threshold for onset of injury in Chinook salmon from exposure to impulsive pile driving sounds. PLoS ONE, 7(6) e38968. doi:10.1371/journal.pone.0038968

Halvorsen, M. B., Casper, B. M., Matthews, F., Carlson, T. J., and Popper, A. N. (2012b). Effects of exposure to pile driving sounds on the lake sturgeon, Nile tilapia, and hogchoker. Proceedings of the Royal Society B. 279, 4705-4714 doi: 10.1098/rspb.2012.154.

Hawkins, A.D., 1986. Underwater sound and fish behavior. In: The Behaviour of Teleost Fishes (T.J. Pitcher ed.) Croom Helm, London, 114-151.

Hawkins, A.D., and Myrberg, A.A. (jnr) (1983) Hearing and sound communication under water. In: Bioacoustics: a comparative approach. B. Lewis (ed.), pp. 347-405. Academic Press, New York.

Hawkins, A. D., Roberts, L. and Cheesman, S. (2014a). Responses of free-living coastal pelagic fish to impulsive sounds, J. Acoust. Soc. Am. 135, 3101-3116.

Hawkins, A. D., Pembroke, A. E. and Popper, A.N. (2014b). “Information gaps in understanding the effects of noise on fishes and invertebrates,” Rev Fish Biol Fisheries DOI 10.1007/s11160-014-9369-3.

Kastelein, R. A., van der Heul, S., van der Veen, J., Verboom, W. C., Jennings, N., Reijnders P., 2007. Effects of acoustic alarms, designed to reduce small cetacean bycatch, on the behaviour of North Sea fish species in a large tank. Marine Environmental Research 64, 160-180.

Kastelein R. A., van der Heul, S., Verboom, W. C., Jennings N., van der Veen, J., de Haan, D. (**2008**). “Startle response of captive North Sea fish species to underwater tones between 0.1 and 64 kHz,” Marine Environmental Research **65**, 369-377.

Lart, B. and Green, K. (2011). Responsible Sourcing Guide: Sea bass. Version 3 – February 2011. Seafish website. http://tinyurl.com/seafishrsg.

Løkkeborg, S., Søldal, A.V., 1993. The influence of seismic exploration with airguns on cod *Gadus morhua* behaviour and catch rates. ICES (International Council for the Exploration of the Sea) Marine Science Symposium 196, 62-67.

Lovell, J.M. 2003. The hearing abilities of the bass, *Dicentrarchus labrax*. Technical report commissioned by ARIA Marine Ltd for the European Commission Fifth Framework Programme. Project Reference: Q5AW-CT-2001-01896

Luczkovich, J.J., Daniel, H.J., III, Hutchinson, M., Jenkins, T., Johnson, S.E., Pullinger, R.C, and Sprague, M.W. (2000) Sounds of sex and death in the sea: bottlenose dolphin whistles suppress mating choruses of silver perch. Bioacoustics**10,** 323–334.

Madsen, P. T. (2005). “Marine mammals and noise: Problems with root mean square sound pressure levels for transients,” J. Acoust Soc. Am. **117**, 3952-3957.

Moulton, J. M., and Backus, R. H., (1955). Annotated references concerning the effects of man-made sounds on the movements of fishes. Fisheries Circ. No. 17, Dep't of Sea and Shore Fisheries, Augusta, Maine.

Myrberg, Jr. A.A., 1990. The effects of man-made noise on the behavior of marine animals. Environment International 16, 575-586.

National Research Council, 2003. Ocean Noise and Marine Mammals. The National Academic Press, Washington D.C., pp 192.

National Research Council, 2005. Marine Mammal Populations and Ocean Noise, Determining when Noise causes Biologically Significant Effects. The National Academic Press, Washington D.C., pp 126.

Nedwell, J.R., Turnpenny, A.W.H., Lovell, J.M., Edwards, B. (2006) An investigation into the effects of underwater piling noise on salmonids. Journal of the Acoustical Society of America 120, 2550–2554.

Nedwell, J.R., Turnpenny, A. W.H., Lovell J. (2007) A validation of the dB ht as a measure of the behavioural and auditory effects of underwater noise. Subacoustech Report No 534R1231

Neo YY, Seitz J, Kastelein R a., Winter HV, Cate C ten, Slabbekoorn H (2014) Temporal structure of sound affects behavioural recovery from noise impact in European seabass. Biol Conserv 178:65–73

Normandeau Associates, Inc. 2012. Effects of Noise on Fish, Fisheries, and Invertebrates in the U.S. Atlantic and Arctic from Energy Industry Sound-Generating Activities. A Workshop Report for the U.S. Dept. of the Interior, Bureau of Ocean Energy Management. Contract # M11PC00031. 72 pp. plus Appendices.

Norro, A. M. J., Rumes, B., and Degraer, S. J. (**2013**). "Differentiating between underwater construction noise of monopole and jacket foundations for offshore windmills: A case study from the Belgian part of the north sea", The Scientific World Journal http://dx.doi.org/10.1155/2013/897624

Popper, A. N., Fay, R. R, Platt, C, and Sand, O. 2003. Sound detection mechanisms and capabilities of teleost fishes. In: Collin, S. P. and Marshall, N. J. (Eds.). Sensory Processing in Aquatic Environments. Springer-Verlag, New York, pp. 3-38.

Popper, A.N., Fewtrell, J., Smith, M.E., and McCauley, R.D., 2004. Anthropogenic sound: Effects on the behavior and physiology of fishes. Marine Technology Soc. J. 37(4):35-40.

Popper, A. N., & Carlson, T. J. (1998). Application of the use of sound to control fish behavior. *Transactions of the American Fisheries Society, 127*, 673-707.

Popper, A. N.., Halvorsen, M. B., Casper, B. M, and Carlson, T. J. (2013). U. S. Dept. of the Interior, Bureau of Ocean Energy Management, Headquarters, Herndon, VA. Effects of Pile Sounds on Non-Auditory Tissues of Fish. OCS Study BOEM 2012-105. 60 pp. OCS Study BOEM 2012-105

Popper, A.N. and Hastings, M.C. (2009). "The effects of anthropogenic sources of sound on fishes," J. Fish Biol.75, 455-89. doi: 10.1111/j.1095-8649.2009.02319.x.

Popper, A.N., Hawkins, A.D., Fay, R.R., Mann, D., Bartol, S., Carlson, T., Coombs, S., Ellison, W.T., Gentry, R., Halvorsen, M.B., Løkkeborg, S., Rogers, P., Southall, B.L., Zeddies, D., Tavolga, W.N. (2014). Sound Exposure Guidelines for Fishes and Sea Turtles: A Technical Report prepared by ANSI-Accredited Standards Committee S3/SC1 and registered with ANSI. ASA S3/SC1.4 TR-2014. Springer and ASA Press, Cham, Switzerland. DOI 10.1007/978-3-319-06659-2.

Rankin CH, Abrams T, Barry RJ, Bhatnagar S, Clayton DF, Colombo J, Coppola G, Geyer M a, Glanzman DL, Marsland S, McSweeney FK, Wilson D a, Wu C-F, Thompson RF (2009) Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiol Learn Mem 92:135–8

Schaefer, K.M. and Oliver, C.W. (1998)."Shape, volume, and resonance frequency of the swimbladder of Yellowfin tuna (*Thunnus albacares*)," SouthWest Fisheries Science Center, Report LJ-98-09C. pp 27.

Yang, J., 1982. The dominant fish fauna in the North Sea and its determination. Journal of Fishery Biology 20, 635-643.