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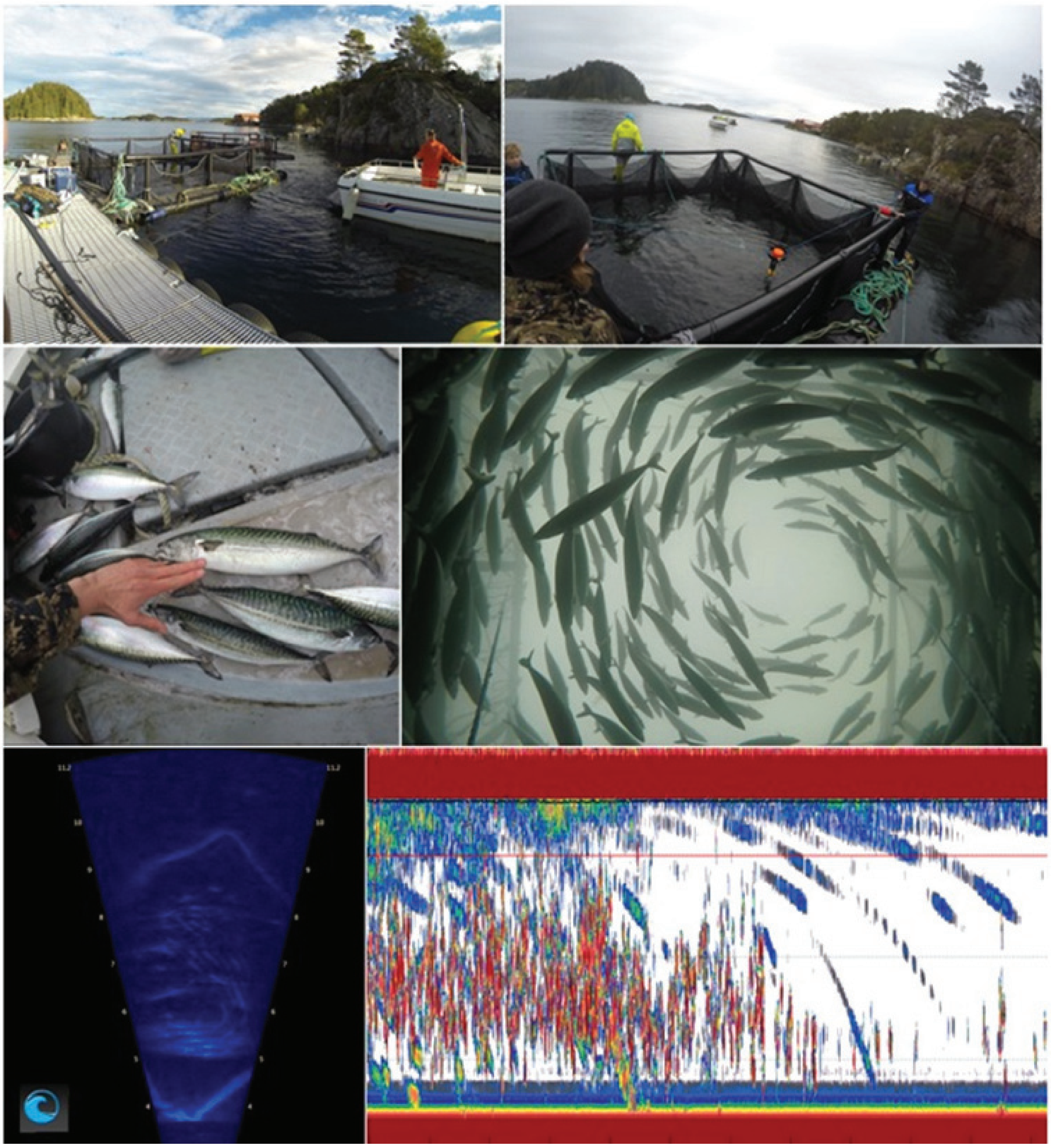
Mackerel behaviour and seismic signals - a pilot net pen study

By Lise Doksæter Sivle, Rune Roland Hansen, Hans Erik Karlsen, og Nils Olav Handegard



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Sammendrag (norsk):

Et pilotprosjekt for å undersøke adferdseffekter av lydeksponering av makrell i merd ble gjennomført høsten 2015 på Austevoll havbrukstasjon. Målsetningen var i første omgang å teste det eksperimentelle oppsettet, samt å undersøke forskjeller i reaksjon til ulike lydkomponenter av et seismisk lydsignal.

Makrell i merd ble eksponert for tilbakespilling av opptak av seismikk skalert i forhold til hverandre for å ha ulik maksimalt trykk og totalt energi innhold, med frekvensområdet under 50 Hz fjernet. I tillegg eksponerte vi dem for to rene toner av ulik frekvens (112 og 14 Hz).

Resultatene viste at makrellen reagert langt sterkest til den rene tonen av 14 Hz, såkalt infralyd. I tillegg til å skille seg fra de andre eksponeringene ved å inneholde slik infralyd hadde denne eksponeringen det høyeste nivået av partikkelakslerasjon av alle eksponeringene. Dette tyder på at lav frekvens og/eller høy partikkelakslerasjon er viktig for å igangsette adferdsresponser hos makrell. Den eksponeringen som gav nest sterkest reaksjon var den med det høyeste energinivået (lyddosen), noe som tyder på at også dette kan være viktig for å indusere adferdsresponser.

Det eksperimentelle oppsettet fungerte svært godt, og video-opptak av adferdsresponser viste seg å være den målemetoden som gav de tydeligste bildene på hvordan en adferdsrespons utartet seg.

Summary (English):
A pilot study to investigate behavioural changes induced by sound exposure for mackerel was conducted in October 2015 at Austevoll research station. The main objective was to test the experimental set up, and secondly to study differences in reactions towards different sound components of a seismic signal.

Mackerel was exposed to playback of recordings of seismic that was scaled differently in terms of peak pressure and total engery content (sound exposure level, SEL), all with frequencies below 50 Hz removed. Additionally, the fish was exposed to two pure tones of 112 and 14 Hz.

Results showed the mackerel to react strongest to the 14 Hz signal. In addition to be the only one of them containing such low frequencies, this exposure also had by far the highest level of particle acceleration. This result indicate that low frequency (infrasound) and/or high particle acceleration is important to trigger a behavioural reaction of mackerel. The second strongest reaction was found towards the exposure with the highest total energy level (SEL), indicating also this to be important to trigger a behavioral response.

The experimental set up used in the pilot worked very well, with the video giving the most clear picture the behavioural response.

Emneord (norsk):	Subject heading (English):
Seismikk, makrell, adferd, merdstudier, mennskeskapt støy i havet	Seismic, mackerel, behaviour, net pen studies, antropogenic noise in the sea

Prosjektleder:	Programleder:
Lise Doksæter Sivle	Frode Vikebø

Contents

1 Background and purpose

Behavioural impact on marine organisms from high-intensity acoustic sources such as pile-driving, sonars and seismic air guns are subject of an increasing concern both among scientists and the general public (Slabberkoorn et al., 2010). Seismic air guns are widely used in the search for, as well as during exploitation of marine oil and gas reserves. Studies have been conducted on the effect of air gun emission on the behaviour of bottom fish, documenting changes in behaviour of caged rockfish (Pearson et al., 1992), horizontal displacement and avoidance by cod and haddock (Engås et al., 1996) as well as changes in the catch composition of various species of bottom fish (Engås et al., 1996; Løkkeborg et al., 2012). Far less knowledge exist on how seismic activities may affect pelagic fish. Slotte et al. (2002) monitored movements of pelagic biomass during a seismic survey, indicating some vertical displacement of herring during the spring migration. Contrary to this, Peña et al (2013) found no response of herring during seismic air gun surveys during the summer feeding period. The last years, there has been repeated claims from fishermen that seismic activity in vicinity of fishing ground of mackerel have caused this species to abandon the area, resulting in lowered catches (e.g. *"I år skal seismikk ikke krasje med makrellfiske"* Fiskeribladet (http://fiskeribladetfiskaren.no/nyheter/?artikkel=32029)).

The main energy of a seismic signal is in the frequency range 20-100 Hz. As sound source distance increases, the energy distribution shifts towards the lower frequencies, as these propagate with less energy loss than higher frequencies. A larger area around the sound source will hence be ensoified by the lowest frequencies. It is well established that many fish species have an extraordinary sensitivity to low frequency sound extending from 50 Hz and well into the infrasonic area, and that sound in this frequency range may elicit natural fright and flight responses in fish (Sand & Karlsen 2000). It may be that mackerel too has a highly developed low frequency hearing, making impulsive sound frequencies below 50 Hz of key importance with respect to evasive behaviours. If so, seismic activity may have the potential to affect mackerel behaviour at distances of hundreds of kilometres from the seismic source.

This is a pilot study to identify the basics of how mackerel may respond to exposure of low frequency sound signals resembling those of seismic signals. Due to this being the first of its kind on mackerel, we do not know how a potential reaction pattern will look like. In a study of free-living mackerel, Hawkins et al. (2014) found mackerel to dive in response to a low-frequency, impulsive sound. Based on several studies on penned herring, we might expect reactions such as diving (Doksæter et al. 2012), changed schooling dynamics such as inter-individual distance or swimming speed (Handegard et al. 2015), or it may change the internal motivation of the fish (Rieucau et al. 2014; 2015). We will try to facilitate for all these type of reactions by having the fish in a deep net pen and proper monitoring equipment to reveal these type of reactions. The experiment in itself will provide valuable results, but the main purpose is to get an idea of the reaction patterns to design solid experiments in the future, as well as determine whether such experiments are appropriate to conduct in sea cages as here, or in situ during a real seismic operation.

The objectives of this study is to test whether a) the fish respond more strongly to the peak pressure of a seismic signal, b) energy content, c) or different temporal structure simulating signal at different ranges, or any combinations thereof. Secondly, we wish to compare the response to a low frequency sound source and playback of the same source, where the playbacks are lacking the low frequency components of the low frequency source, investigating the importance of frequency components lower than that of the playback loop is capable of.

A laboratory study of mackerel hearing was also conducted at University of Oslo in authum 2015, and these two experiments are complimentary to each other.

2 Methods

2.1 Experimental location

The experiments were conducted at the Institute of Marine Research (IMR)s research facility Austevoll Havbruksstasjon outside Bergen, Norway.

Free living mackerel had been captured throughout the summer of 2014 and 2015, and held in large net pens (10x10x10m).

For the purpose of the current experiment, we had available two smaller, square net pens (5x5x5m), that easily can be towed from a small boat. These have been used in earlier experiments (see Doksæter et al. 2012) for similar work with herring. With two different, moveable pens available, we could efficiently change experimental fish, thus avoiding habituation to a large extent.

From the large net pens at the main station, a small amount of fish (about 200-300 individuals) were transferred each day to one of the smaller, experimental net pens. This pen was than towed to the experimental location (see figure 1). The two identical net pens, allowed for a very efficient use of time. In the afternoon, fish was transferred to one of the pen at location A, and acclimatized over night. In early morning, this pen was towed slowly to location B, and mounted to the floating pier. The pen already here from the previous day was than towed back to A, were the fish was humanely killed. After the pen was empty, new fish was transferred to be ready for the next day, and the process repeated.

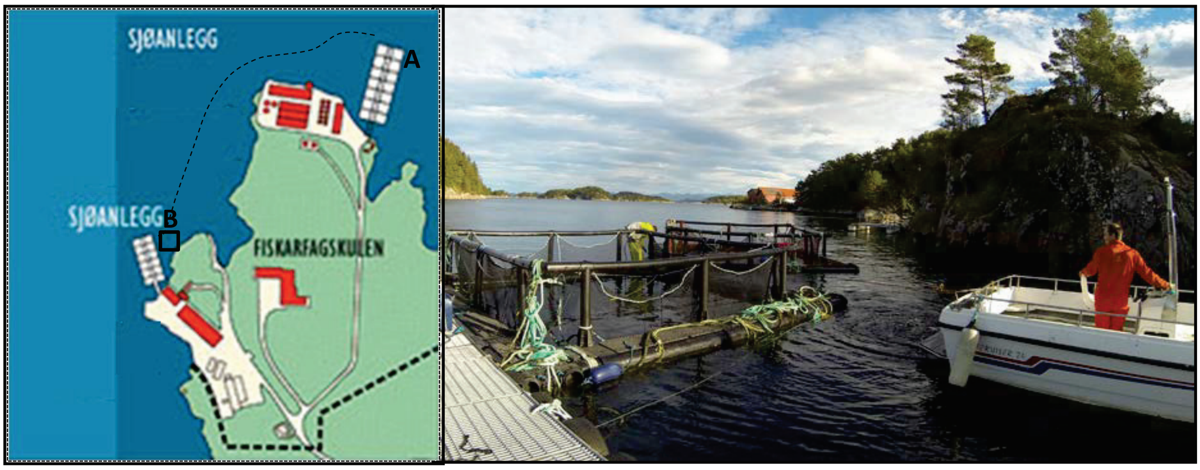

Figure 1. Left: Overview of experimental location at Austevoll Havbrukstasjon. Fish will be held at location A, and towed in a net from A to B (indicated by stippled line) prior to experiment. Experiments was be conducted at location B. Rigth: Fish transfer in progress at location B. Every morning, a new batch was towed from A to B, and the two pens switched, with the old fish being towed back and humanely killed.

Two sound sources as well as monitoring equipment for behaviour and sound exposure were placed inside and outside the pen. The two sound sources were controlled from an onshore shelter, while the monitoring equipment, due to lower cable length, were control from a small boat docked close to the pen. Details of the experimental set up are shown in figure 2 and explained below.

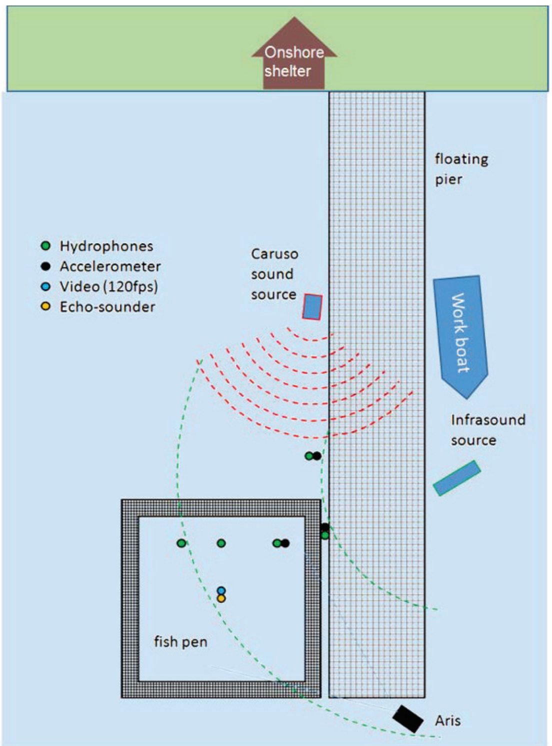

Figure 2. Sketch of experimental set up. The fish pen was moored to a floating pier. The two sound sources , Caruso and Infrasound source was placed 7 and 2 m from the pen, respectively. Two hydrophones were always placed inside the pen, at different depths; 1 and 3 m. The third hydrophone were placed together with the particle motion sensor at three different locations, inside the corner of the pen, just between the infra-sound source and the pen, and between the Caruso source and the pen.
Hydrophones, echosounder and ARIS was mounted to be controlled from a small workboat docked at the pier, allowing indoor operation during rain.
Both sound sources were controlled from a small shelter on land.

2.2. Monitoring behaviour in the pen

Fish behaviour was monitored by echosounder, high frequency sonar (ARIS) and video camera. The upward looking 120 kHz split-beam echosunder (Simrad EK 60, Kongsberg Maritime AS, Horten, Norway) was placed in a gimble rigg at the bottom end of the pen, at 4.5 m depth (figures 3, 4). A horizontally-looking high-frequency imaging sonar, ARIS (Sound Metrics, Washington, USA), operating at 1.8 MHz and recording at 8 frames/s, was deployed on one side of the sea-cage at approximately 2 m depth and 3 m from the net pen wall (figure 2). The sea-cage wall was within the field of view at the opposite end.

Both echosounder and ARIS was controlled and monitored from the workboat, allowing real-time monitoring of behaviour inside the pen (figure 5).

The video camera, GoPro Hero4 black edition, was set to record high definition video (1080p, 1080x1920 pixels) at 120 frames per second and mounted on the gimble rig next to the echosounder, facing upwards to film the above schooling mackerel.. The experimental set up allow for monitoring and recording different scales of behaviour; from large scale collective vertical movements to fine scale schooling dynamics such as swimming speed and inter-individual distance.

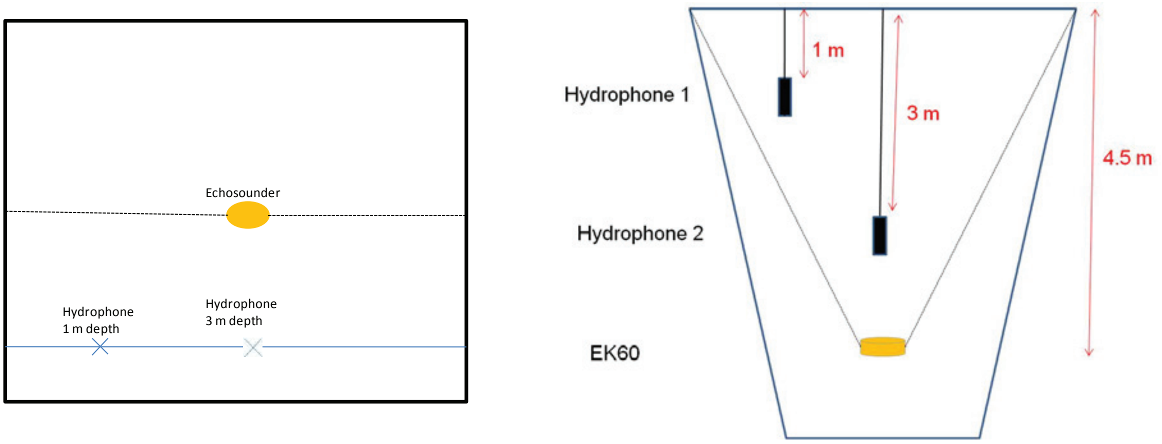

Figure 3. The net pen seen from above (left) and from the side (right). Hydrophones were placed at 1 and 3 m depth, and the echosounder (EK60 120 kHz), looking upward, was placed in the middle of the pen at 4.5 m depth, thus covering the most of the net pen. The go-pro camera was placed at the same rig as the echosounder.

Figure 4. Left: Net pen with the echosounder at depth, visible as a orange ring. Placement of the two sound sources and the workboat were recording or echosounder, ARIS and hydrophone data were done. Rigth: The echosuder was mounted in a gimble ring, to make it stable under water. Here liftet to the surface. The go-pro camera was also mounted on this ring.

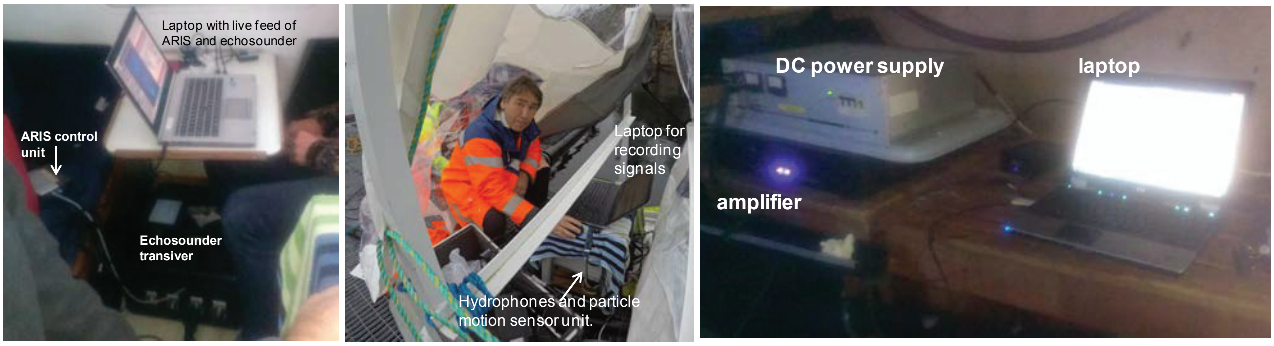

Figure 5. Controlling the different units. Left: Echosounder and ARIS was controlled and monitored from inside the workboat. Middle: Due to the short cable of the particle motion sensor, the hydrophone and particle measurements was recorded at the dock just next to the pen. When not recording particle motion, this was also done from the workboat. Right: Sounds were played from a laptop connected to an amplifier and DC power source that again was connected to the underwater sound source.

2.3 Monitoring sound in the pen

The sound pressure in the pen was monitored using a pair of calibrated Brüel & Kjær (B&K) type 8106 hydrophones pre-amplified with a B&K Nexus conditioning amplifier. These were mounted at the end of the pen at 1 and 3 m depth (figure 3). The hydrophones were calibrated on-site using a B&K Type 4229 hydrophone calibrator with a B&K WA0658 coupler. A full factory calibration was performed in 2012, and, using the calibrator, the on-site calibrations were compared to those taken immediately after the factory calibration.

Acoustic particle accelerations were measured by a small sized (≈ $1cm^3$) linear accelerometer type Entran EGCS-A2-2 (Entran Devices, Inc., Fairfield, NJ, USA) placed in the center of a Plexiglas cylinder (diameter and length 5cm) adjusted to have the same overall density as the sea water at the test site. The working range of the accelerometer was ± 2g, corresponding to a sensitivity of approximately 0,25 $Vm^{-1}s^2$. The accelerometer unit was suspended by a fine nylon thread and operated at recording positions 1m outside and 1m inside the net pen respectively and at the same time directly in front of and directly in line with the working axis's of the sound sources. The alignment of the accelerometer with respect to the sound sources was determined visually while horizontal alignment was monitored by the DC-output from the accelerometer. Overall the accelerometer unit thus measured maximum particle accelerations in the horizontal plane in front of the sound sources 1m outside and inside of the net pen.

The digitized hydrophone data were imported to Matlab (Mathworks, Inc.) for further analysis. The first step was to convert the signal from voltage to pressure using the calibration constant from the on-site calibration resulting in a pressure time series for each playback. For each playback, the maximum pressure and the sound exposure level (SEL) was calculated. The SEL was calculated by taking 10*log of the integrated the pressure squared over the duration of the pulse.

2.4 Sound sources and sound stimuli

The fish was exposed to five different sound stimuli, three playbacks of seismic signals, and two pure tone signals at two different frequencies.

The seismic pulses used in the playbacks were recorded during an experiment outside Lofoten and Vesterålen (Hovem et al., 2012; Løkkeborg et al., 2012). Two recordings taken at different ranges were used, one at a distance about 8 km and one at 120 km, respectively. The signal at long range has a different structure than that of a close range, most notably that the energy is distributed wider temporally compared to that of close range (figure 6). This temporal dilution of the signal is caused by frequency dependent wave speeds and the signal taking different paths (Hovem et al., 2012). One of the questions is whether this temporally different structure causes different fish behaviour, indicating that a sharp pulse at short range is more disturbing that at range.

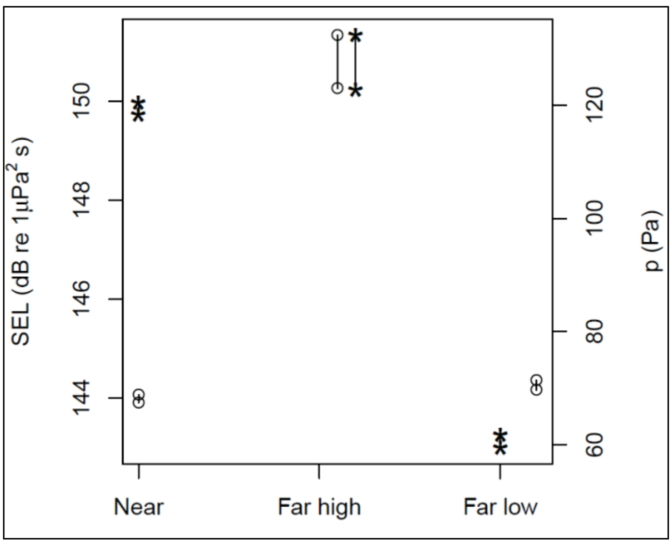

Figure 6. The measured sound exposure level (SEL), indicated with circles, and peak pressure (p) levels, indicated by asterisk, in the pen for the three different seismic playbacks. Two recordings are here shown, from two different blocks (block 1 and 2), indicating that even though it is the same signal that is played, there may be some small differences in the received signals between blocks.

Detailed characteristics of the three seismic playback signals:

Seismic close - Seismic signal recorded at distance about 8 km from the source. The signal had SEL=144 dB (re 1µPa^2 s) and peak pressure ~118 Pa.

Seismic far low - Seismic signal recorded about 120 km from the source. The signal had peak pressure of 59 Pa, thus lower than the near signal, and were adjusted to have similar SEL to the close signal (144 dB (re 1µPa^2 s)).

Seismic far high - The same signal as seismic far low, but with adjusted peak pressure to be in the similar range as seismic near (~122 Pa) and SEL=151 dB (re 1µPa^2 s), thus higher than the other two signals.

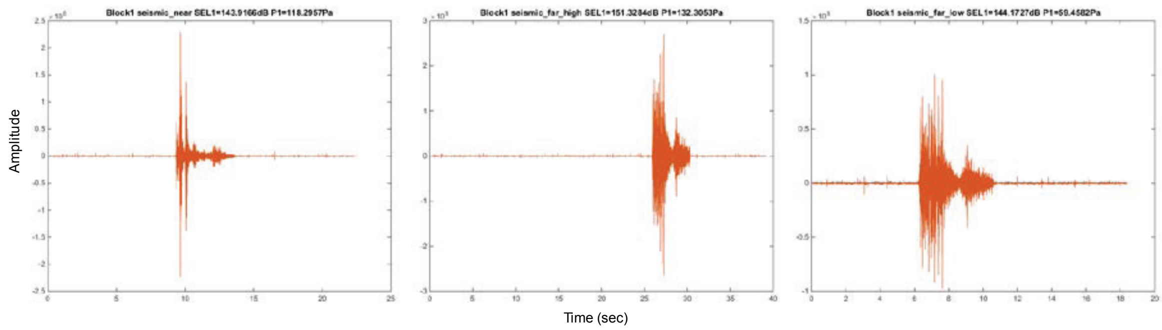

Figure 7. Characteristics of the three seismic playback signals; seismic near (right panel), seismic far high (middle panel) and seismic far low (left panel). Note the different scales on both the x and y axes.

Details of the two pure tone signals:

Infrasound - A pure 14 Hz pulse of ~ 3 sec duration, hereafter referred to as 14Hz. This is representative of the lowest spectre of the seismic signal, and the part of the signal that has the longest range due to the low absorption at such low frequencies. The SEL and peak pressure of the signal was 145 dB (re 1µPa^2 s) and 34 Pa, respectively.

Higher frequency - This was a recording of the infrasound signal played 6 times faster, thus 112 Hz, hereafter referred to as 112 Hz. The signal was amplified to get the same SEL (~145 dB (re 1µPa^2 s)) and peak pressure (~34 Pa), and had the same duration (3 sec). The two signals differed thus mainly in their frequency content.

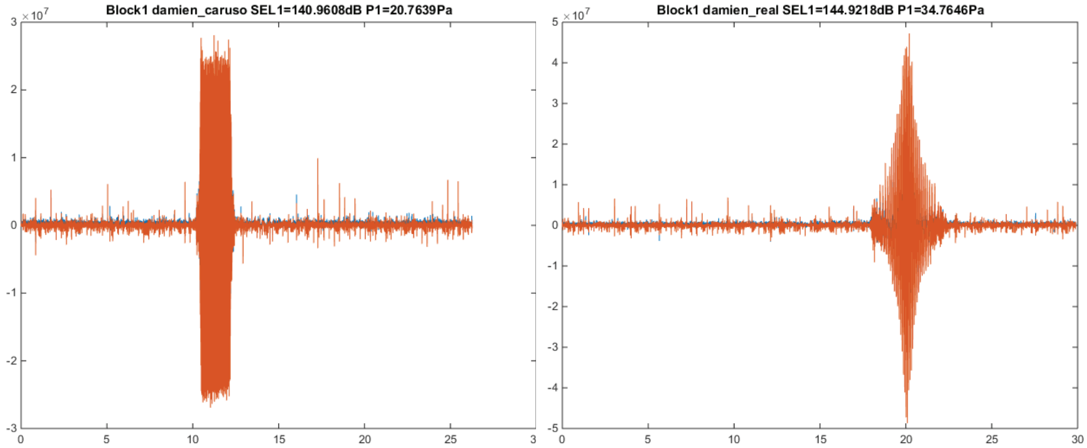

Figure 8. Characteristics of the two pure tone signals; 112 Hz (right panel) and infrasound (left panel). Note the different scales on both the x and y axes.

We used two different sound sources, a high energy transducer (Caruso) and a single frequency low frequency source (Infrasound source).

The high energy transducer was used to play back the seismic signals and the recording of the infrasound source. Prior to playback, the recorded seismic signal was filtered to remove noise using a low pass finite impulse response filter (*firpmord, Fpass=500 Hz, Fstop=800 Hz*). The signal was played back through a Hegel HD2 High End USB Music streamer device modified to produce waveforms down to 5 Hz. The sound card was connected to power amplifier (Cerwin-Vega CXA-10) an underwater transducer. The transducer (similar to that used by (Engås *et al.*, 1995; Handegard *et al.*, 2014)) was based on a moving coil acting against an electromagnet that drove a membrane of 0.3 m in diameter. To drive the electromagnet in the transducer, a SMPS switch mode DC power supply providing up to 230V at 6 A was used. The maximum continuous power rating was 0.5 kW for the moving coil and 1.5 kW for the electromagnet. The sound was played from a laptop computer located together with the power amplifier and DC power supply in the onshore shelter (figure 5)

The infrasound source (Pro fish Technology, Belgium) generates the signal by two rubber membranes attached to two pistons that are driven 180 degrees out of phase. The rubber membranes have a diameter of 25 cm each, and the peak-to-peak amplitude of the piston movements was 5·0 cm. The pistons were coupled to a crankshaft driven by a brushless 1.5 kW servomotor, and resulted in changes in water volume similar to a monopole source (Sonny *et al.*, 2006).

2.5 Experimental protocol

The experiment used a block design, with each block consisting of the five different treatments (14Hz, 112Hz, seismic far low, seismic far high, seismic close) presented in a randomized order. Exposures were randomized prior to each block.

Blocks were separated by minimum 1 hour, and the sound stimuli within each block were presented minimum 5 min apart. Before each block 10 min of baseline behaviour was recorded.

Each block were conducted by at least three people. One were responsible to synchronization of timing an signalling onset of stimuli as well as operating the EK60 and ARIS. This was localized in the work boat. The second were responsible to logging of hydrophone and particle measurements. The third person was responsible doing the sound playback, done from either the infrasound source or from a laptop connected to the Caruso source (fig. 5).
If some additional external disturbance occurred just prior to onset of a stimuli, e.g. a boat passing close by, we waited additional 5 min before stimuli onset.

3 Data collected

3.1 Overview

A total of 4 batches of fish was used over four succeeding days, with a total of 10 blocks (table 1).

The four batches of fish contained 254, 315, 420 and 550 fish, respectively. Fish had a mean length of 39.9 cm and weigh of 897.8 g.

We have mainly used the Go Pro video and echosounder data to evaluate fish behaviour. The ARIS data were used as support to the reactions seen in video and echosounder.

Table 1. Overview of experiments.

Mackerel batch	Date	Block	Stimulus order	Stimulus type	Time start log	Time start stimuli
batch1	06.10.2015	1	1	Infrasound	15:01:00	15:01:18
batch1	06.10.2015	1	2	seismic_far_low	15:14:00	15:05:12
batch1	06.10.2015	1	3	seismic_near	15:21:00	15:12:15
batch1	06.10.2015	1	4	seismic_far_high	15:28:00	15:28:23
batch1	06.10.2015	1	5	Higher freq	15:33:00	15:33:08
batch1	06.10.2015	2	1	seismic_far_high	18:02:00	18:02:04
batch1	06.10.2015	2	2	Higher freq	18:08:00	18:08:08
batch1	06.10.2015	2	3	seismic_near	18:14:00	18:14:03
batch1	06.10.2015	2	4	seismic_far_low	18:20:00	18:20:08
batch1	06.10.2015	2	5	Infrasound	18:28:00	18:29:00
batch2	07.10.2015	3	1	seismic_near	11:10:00	11:10:23
batch2	07.10.2015	3	2	Higher freq	11:16:00	11:16:13
batch2	07.10.2015	3	3	seismic_far_low	11:22:00	11:22:06
batch2	07.10.2015	3	4	seismic_far_high	11:28:00	11:28:06
batch2	07.10.2015	3	5	Infrasound	11:35:00	11:35:06
batch2	07.10.2015	4	1	Higher freq	13:32:00	13:32:09
batch2	07.10.2015	4	2	seismic_far_low	13:38:00	13:38:09
batch2	07.10.2015	4	3	Infrasound	13:44:00	13:44:14
batch2	07.10.2015	4	4	seismic_near	13:53:00	13:53:06
batch2	07.10.2015	4	5	seismic_far_high	13:59:00	13:59:06
batch2	07.10.2015	5	1	Infrasound	15:45:00	15:45:10
batch2	07.10.2015	5	2	seismic_near	15:54:50	15:54:58
batch2	07.10.2015	5	3	seismic_far_high	16:00:50	16:00:57
batch2	07.10.2015	5	4	Higher freq	16:05:57	16:06:04
batch2	07.10.2015	5	5	seismic_far_low	16:12:00	16:12:08
batch 3	08.10.2015	6	1	Infrasound	10:36:30	10:36:38
batch 3	08.10.2015	6	2	seismic_far_low	10:51:01	10:52:05
batch 3	08.10.2015	6	3	seismic_far_high	11:07:00	11:07:02
batch 3	08.10.2015	6	4	Higher freq	11:13:00	11:13:03
batch 3	08.10.2015	6	5	seismic_near	11:19:00	
batch 3	08.10.2015	7	1	seismic_far_low	12:38:00	12:38:38
batch 3	08.10.2015	7	2	Higher freq	12:43:10	12:43:15
batch 3	08.10.2015	7	3	seismic_near	12:48:00	12:48:03
batch 3	08.10.2015	7	4	Infrasound	12:53:00	12:53:09
batch 3	08.10.2015	7	5	seismic_far_high	13:10:15	13:10:24
batch 3	08.10.2015	8	1	seismic_far_high	14:13:15	14:13:19
batch 3	08.10.2015	8	2	Infrasound	14:19:00	14:19:08
batch 3	08.10.2015	8	3	seismic_near	14:25:00	14:25:06
batch 3	08.10.2015	8	4	Higher freq	14:30:00	14:30:05
batch 3	08.10.2015	8	5	seismic_far_low	14:35:30	14:35:34
batch 4	09.10.2015	9	1	Higher freq	08:17:00	08:17:06
batch 4	09.10.2015	9	2	seismic_far_high	08:22:00	08:22:07
batch 4	09.10.2015	9	3	seismic_far_low	08:28:15	08:28:20
batch 4	09.10.2015	9	4	Infrasound	08:34:15	08:34:21
batch 4	09.10.2015	9	5	seismic_near	08:42:00	08:42:07
batch 4	09.10.2015	10	1	Infrasound	09:51:00	09:51:11
batch 4	09.10.2015	10	2	seismic_far_low	09:57:00	09:57:07
batch 4	09.10.2015	10	3	Higher freq	10:03:00	10:03:04
batch 4	09.10.2015	10	4	seismic_near	10:10:30	10:10:34
batch 4	09.10.2015	10	5	seismic_far_high	10:18:00	10:18:04

3.2 Sound exposure in pen

The resulting sound characteristics of the 5 treatments are shown in Table 2. The experiment was designed to separate between frequency, peak pressure and SEL. In addition, we were able to measure particle acceleration.

Table 2. Signal characteristics of the 5 different sound treatments.

Treatment	Sound exposure level (SEL) [dB(re 1µPa2 s)]	Peak preassure (Pa)	Frequency (Hz)	Particle acceleration (ms^{-2})
14Hz	145	34	14	0,03
112Hz	145	34	112	0,007
Seismic close	144	118	50-200	0,004
Seismic far low	144	59	50-200	0,002
Seismic far high	151	122	50-200	0,004

3.3 Echosounder data

The echosounder recorded the acoustic volume backscatter strength by time and depth at a sampling frequency of 1 Hz over a range from its position at 4.5 m depth to the surface. Volume backscattering strength is defined as $sv = \sum \sigma_{bs} / V$ (m^{-1}), where V is volume, and bs is the backscattering cross sections of individual targets within V (definitions given in MacLennan *et al.*, 2002).

Typical echograms for each of the 5 treatments are shown in Figure 9.

Two response variables, *depth* and *sv*, are derived from the data. The variable *sv* is defined as the mean volume backscattering over the vertical window spanning from 0.75 m from the echousounder up to about 0.5 m from the surface (Figure 9), and *depth* is defined as the *sv* weighted median depth. For each exposure, data are given as the average for three time intervals; 15 - 5 seconds prior to exposure (pre), from start of exposure and 10 sec onwards (dur) and 15-25 sec after start of exposure (post) (Figure 9). Mean and standard deviations are shown in table 3, and shown graphically in Figure 10.

Table 3. Mean ± standard deviation for the variables depth and sv for three defined times periods Pre, Dur and Post.

	depth			*sv*		
	Pre	Dur	Post	Pre	Dur	Post
112Hz	1.87 ± 0.45	1.78 ± 0.5	1.81 ± 0.5	$4.48*10^{-5} \pm 3.95*10^{-5}$	$7.24*10^{-5} \pm 1.14*10^{-5}$	$8.12*10^{-5} \pm 6.45*10^{-5}$
14Hz	2.05 ± 0.35	1.89 ± 0.26	2.03 ± 0.33	$6.07*10^{-5} \pm 5.77*10^{-5}$	$5.91*10^{-5} \pm 4.03*10^{-5}$	$3.78*10^{-5} \pm 5.11*10^{-5}$
seismic close	1.89 ± 0.35	1.96 ± 0.38	1.81 ± 0.26	$4.40*10^{-5} \pm 3.40*10^{-5}$	$2.34*10^{-5} \pm 2.0*10^{-5}$	$3.15*10^{-5} \pm 3.91*10^{-5}$
seismic far high	1.94 ± 0.38	1.90 ± 0.42	1.80 ± 0.42	$4.81*10^{-5} \pm 3.84*10^{-5}$	$3.82*10^{-5} \pm 2.95*10^{-5}$	$4.46*10^{-5} \pm 4.26*10^{-5}$
Seismic low	2.00 ± +.57	1.95 ± 0.48	1.92 ± 0.55	$4.78*10^{-5} \pm 4.22*10^{-5}$	$3.05*10^{-5} \pm 3.21*10^{-5}$	$3.11*10^{-5} \pm 3.56*10^{-5}$

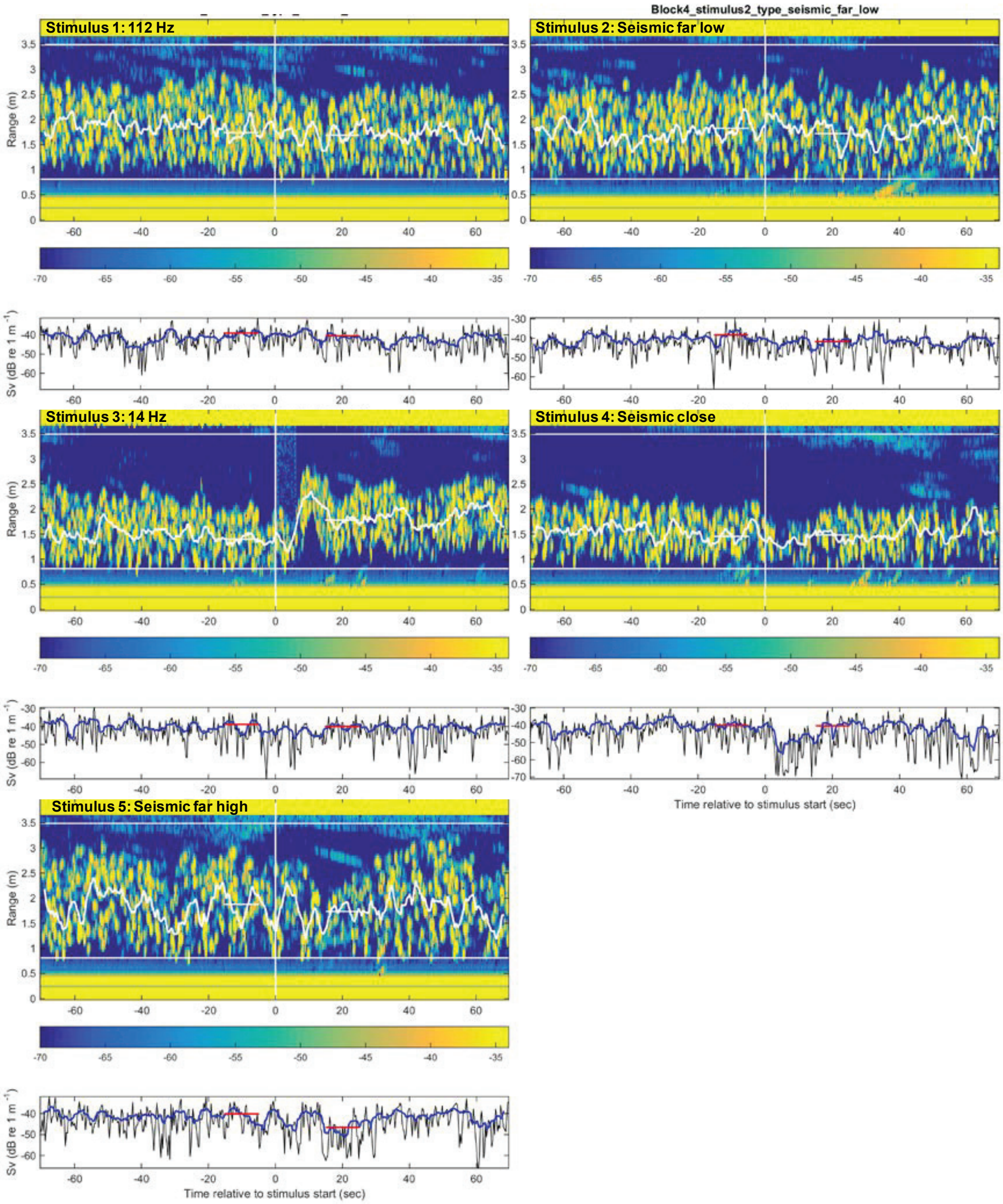

Figure 9. Example of echogram and data for *sv* and *depth*. The upper panel of each figure show the echogram with the white line showing the depth variable. The echosunder is placed at 4.5 m depth, given as 0 on the y axis. The lower panel of each figure show the *sv*. Time is given with time=0 is tart of the stimulus. Thus the average *sv* and *depth* is calculated for time intervals from -15 to -5 (Pre), 0 to 10 (Dur) and 15 to 25 (Post). Pre and post periods are marked in the figure. This example echograms are for block 4 (second block of batch 2), and the treatments are presented in the order used in this block.

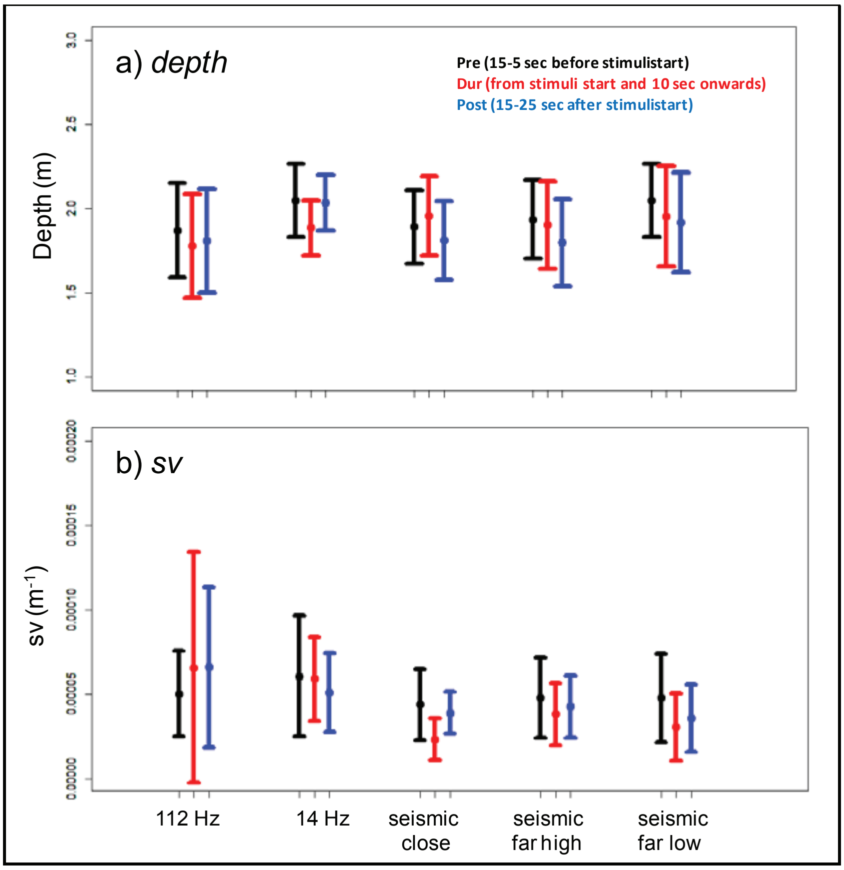

Figure 10. Mean and standard deviation for a) *depth* and b) *sv* for the five different treatments for the periods defined as Pre, Dur and Post.

3.4 Video data

Video recordings were used to analyse behavioural responses in two ways: 1) quantitatively - measuring swimming speeds before, during and after sound stimulus, and 2) qualitatively - scoring the observed behavioural responses.

Analyses of swimming speed

Sequences of still frames, starting 30 seconds before, to 30 seconds after sound exposures, were exported from the video files using the video editing software, Adobe Premiere Pro CS5.5. The image tracking software, ImageJ (plugin MTrackJ), was used to track individual fish in three sequential frames (time step - 1/15 second per frame), enabling two distances of movement calculated per fish (in pixels), which in turn was converted into swimming speeds. The mean body length of the experimental fish was 40 cm (as measured in 120 mackerel of the first batch), and this was used as a standard measure for transforming pixel values from the tracking operation to measures in meters. Swimming speeds (m/s^2) were calculated as following:

$$\frac{Mean\ body\ length;\ i.e.\ 40\ cm}{Measured\ body\ length\ (pix)} * Distance\ tracked\ (pix) =$$

$$\frac{Distance\ tracked\ (cm)}{15\ frames*sec-1} *15 * 1/100 = \underline{Swimming\ speed\ (\mathrm{m/s^2})}$$

Selection of fish for tracking was based a strict set of criteria to ensure comparability of the swimming speed measurements:

- The fish had to be within a fixed centre section of the frame, to minimize the effect of image distortion due to the wide angle camera lens (i.e. fish eye effect).

- The fish body had to be straightened out in one of the three frames to enable appropriate body length measurement.

- The fish was not undertaking conspicuous foraging behaviour (i.e. snapping), since this might have influenced swimming speeds significantly.

- The fish were of “medium” and similar size classes.

The mean of the two swimming speeds attained per fish was then used to calculate the mean swimming speeds from a minimum of five fish, in a given period. Mean swimming speeds were measured in three phases; Pre exposure (30, 20, 10 and 1 sec before stimulus onset), During exposure (1, 2 and 3 sec after onset) and Post exposure (5, 10, 20, and 30 sec after ended exposure). The data are summarised in table 4, and plotted in figure 13.

Table 4. Mean swimming speeds (ms^{-1}) ± standard deviations for the different treatments and times relative to start of exposure (start at time=0), colours indicating experimental phases; Pre (green), During (red) and Post exposure (blue). Differences in post exposure times owe to differences in signal durations; i.e. 14Hz and 112Hz were approximately 1 sec longer and 2 sec shorter respectively, than the three seismic signals.

Time (sec relative to stimulus onset)	14Hz	112Hz	Seismic far low	Seismic far high	Seismic close
-30	0.48 ± 0.05	0.46 ± 0.03	0.49 ± 0.06	0.49 ± 0.06	0.47 ± 0.05
-20	0.48 ± 0.04	0.45 ± 0.04	0.48 ± 0.07	0.47 ± 0.05	0.48 ± 0.06
-10	0.48 ± 0.05	0.45 ± 0.03	0.49 ± 0.05	0.47 ± 0.05	0.46 ± 0.05
-1	0.48 ± 0.06	0.46 ± 0.04	0.49 ± 0.05	0.48 ± 0.07	0.48 ± 0.06
1	0.48 ± 0.05	0.49 ± 0.05	0.49 ± 0.04	0.56 ± 0.19	0.51 ± 0.09
2	1.04 ± 0.57	-	0.51 ± 0.06	0.55 ± 0.08	0.53 ± 0.09
3	-	0.51 ± 0.06	-	-	-
7	-	0.51 ± 0.07	-	-	-
9	0.54 ± 0.1	-	0.49 ± 0.06	0.48 ± 0.07	0.48 ± 0.08
12	-	0.49 ± 0.07	-	-	-
14	0.54 ± 0.11	-	0.48 ± 0.07	0.47 ± 0.03	0.49 ± 0.07
22	-	0.49 ± 0.05	-	-	-
24	0.52 ± 0.1	-	0.48 ± 0.07	0.47 ± 0.04	0.49 ± 0.06
32	-	0.48 ± 0.06	-	-	-
34	-	-	0.47 ± 0.07	0.47 ± 0.06	0.47 ± 0.04
35	0.49 ± 0.08	-	-	-	-

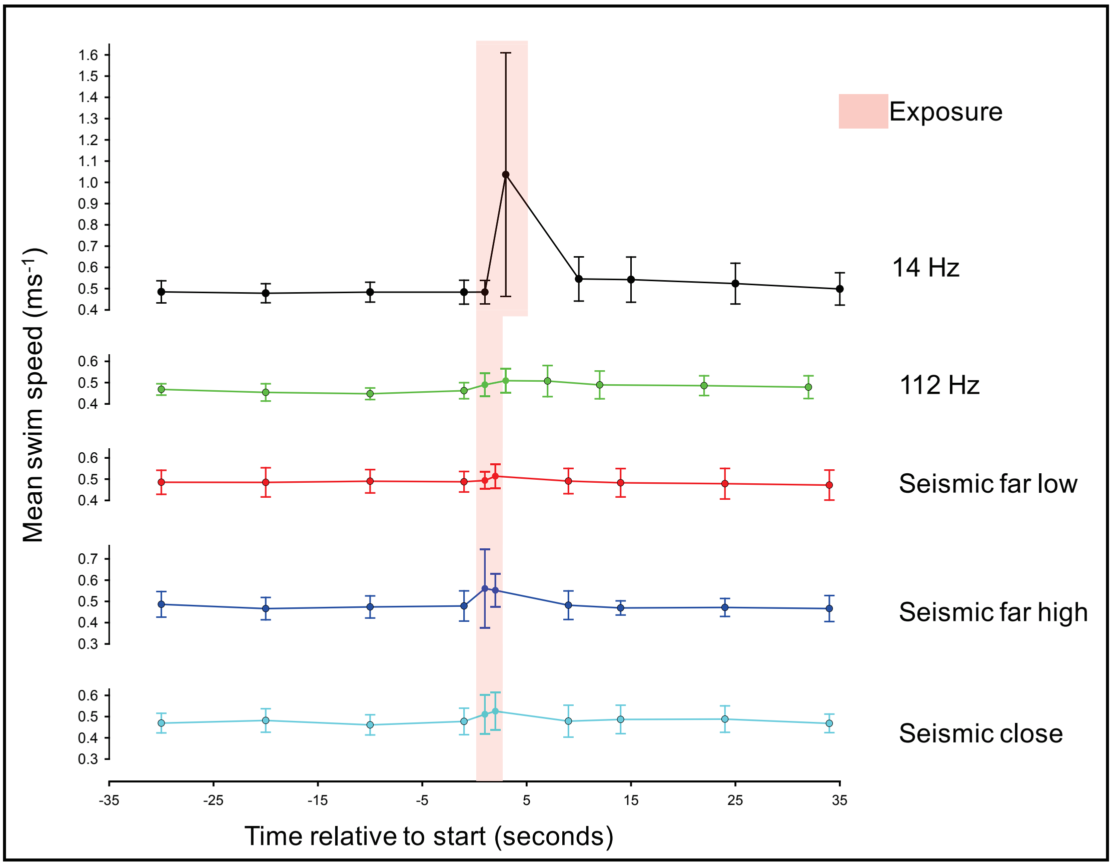

Figure 13. Mean swimming speeds and standard deviations per time stages relative to stimulus onset (t=0). The red bar show the approximate duration of the exposure.

Video scoring

Qualitative analysis by scoring of behavioural responses in the videos was done by two behavioural experts using a predetermined set of scoring rules, scoring three different behavioural traits (startle response, swimming speed and collective behaviour) on a scale of 0-3 (table 5). The two scorers (authors LDS and RRH) scored the videos from each run independent of each other, and the scoring was done without sound, so the exposure sound was not influencing the evaluation. Following this, scoring for the two were compared, and for cases with disagreement of scoring, discussed and evaluated for consensus.

Table 5. Definitions of scorings used for scoring responses of video.

Score	Category A: Startle	Category B: Swimming speed	Category C: Collective behaviour	Number of fish reacting	Duration of reaction (applicable to B and C)
0	No response	No change in swimming speed	No visible change	None	-
1	Weak startle, but no clear S or C-start	Weak change in swimming speed	Weak change in coordination between individuals. Closer toghether and more coordinated, but few fish and short duration.	<5 individuals	Shorter than exposure
2	Presence of S-start.	Moderate increase in swimming speed, caracterized by a clear increase, that drop again relatively rapidly.	Moderate change in coordination.	>5 individual	Approximately the duration of the exposure.
3	Presence of C-start.	Strong increase in swimming speed, lasting after end of exposure.	Strong change in coordination. Fish becomre more aligned, lasting after ended exposure	most in visible range	Longer than duration of exposure.

Of the total of 150 scorings (50 treatments, three categories of scoring of each) only 6 were subject to disagreement. After discussion and re-inspection of video, consensus scoring was agreed upon. Scorings of category B, swimming speed, were in strong agreement with the quantitative measurement of swimming speed; i.e. runs with a high increase in swimming speed also received a high scoring (score 2 or 3), thereby strengthening the confidence of the scoring. Mean and standard deviations for the three categories are shown in table 6.

Table 6. Mean ± standard deviation for video scoring for the different scoring categories and treatments.

	Category A: Startle	Category B: Swimming speed	Category C: Collective behaviour
112 Hz	0.22 ± 0.67	0.33 ± 0.71	0.22 ± 0.67
14 Hz	2 ± 0.94	2.10 ± 0.74	1.7 ± 1.16
Seismic close	0.33 ± 0.71	0.56 ± 0.73	0.44 ± 1.01
Seismic far high	1 ± 0.87	0.89 ± 0.78	0.22 ± 0.44
Seismic far low	0 ± 0	0.1 ± 0.32	0 ± 0

4 Results

4.1 Echosounder and swimming speed data

For swimming speed, there was a clear trend of increase in swimming speed during the 14 Hz treatment (figure 13). For the echosounder data, there was a trend of decreased depth during 14 Hz exposure and decreased sv during seismic close (figure 10).

We are mainly interested in understanding how the fish respond to the various sound treatments, and the difference in response between treatments. However, several other factors may influence the results, and it is important to understand how these factors influence the variability of the dataset. This may help us interpret the results.

Data were analyzed using linear mixed models, using the packages *lm* and *lme* in R.

For the variables swimming speed, depth and sv, the response is described in term of the change from the pre exposure level to the exposure level by introducing a dummy variable that is one during exposure and zero before.

Data may be influenced by factors block, batch and test order. First we looked for trends in the ”pre dataset”, defined as the baseline fish behavior prior to exposure) and how that was influenced by these factors. This is shown in figure 14.

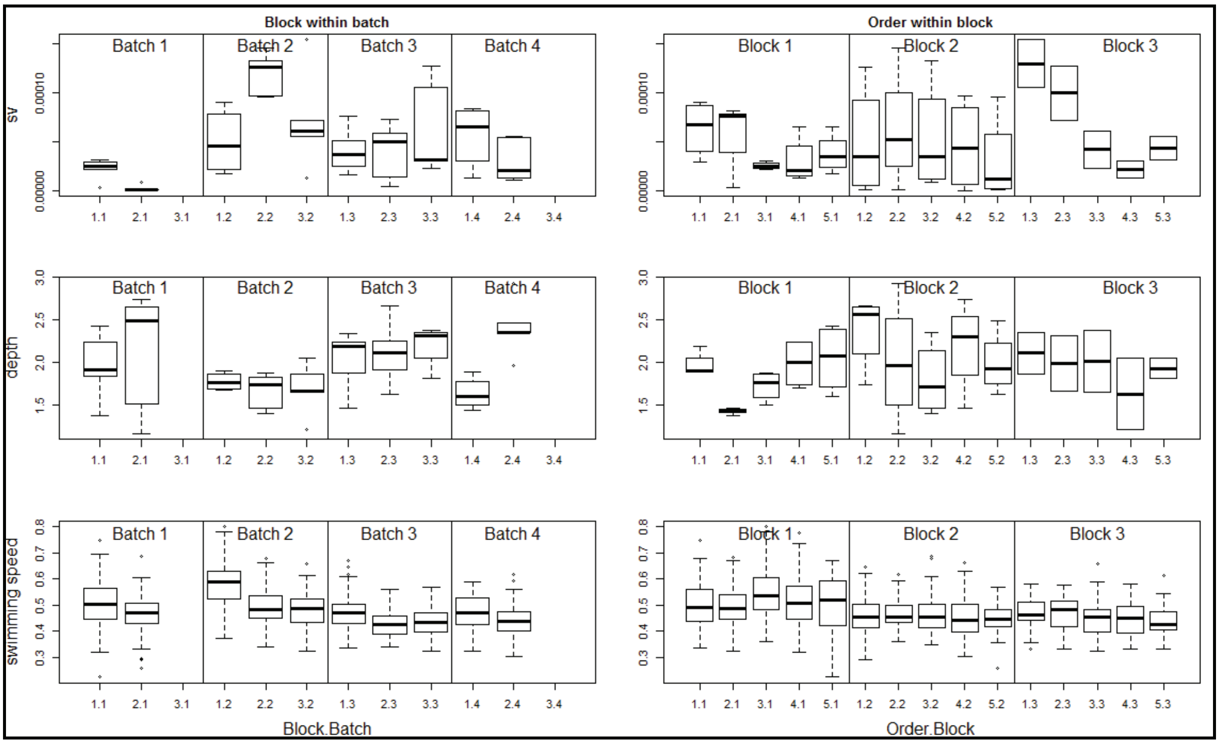

Figure 14. Influence of order, block and batch on the explanatory variables prior to exposure. The figure shows the variation of the three different blocks within the four batches (left) and test order within block (rigth) for the three different response variables sv (upper panel), depth (middle panel) and swimming speed (lower panel).

The pre data were modeled with order, block and batch all as fixed effects, and significant effects were found for batch, order and block.

For *swimming speed*, the block number was the most important, with decreasing swimming speed for succeeding blocks within each batch (figure 1 left lower panel). . There were also significant, but weaker effects of test order and batch number.

For *depth*, batch was most important, with fish batch 2 having a deeper vertical distribution than the remaining three blocks. For *sv*, batch was most important, also with batch 2 standing out with significantly higher sv than the rest. Particular block 2 in batch 2 seems to be high (figure 1)- Significant block effects in pre data could be interpreted as changes throughout the day or that repeated exposures changes the structure of the school within the pen.

Analyzing the response

Our key interest is the change in the response variables (swimming speed, depth, sv) in response to the exposures, estimated by comparing the Pre exposure period to the exposure in response to the different treatments (Figure 15). This was achieved by introducing a binary dummy variable, termed binary.response, representing the response during exposure.

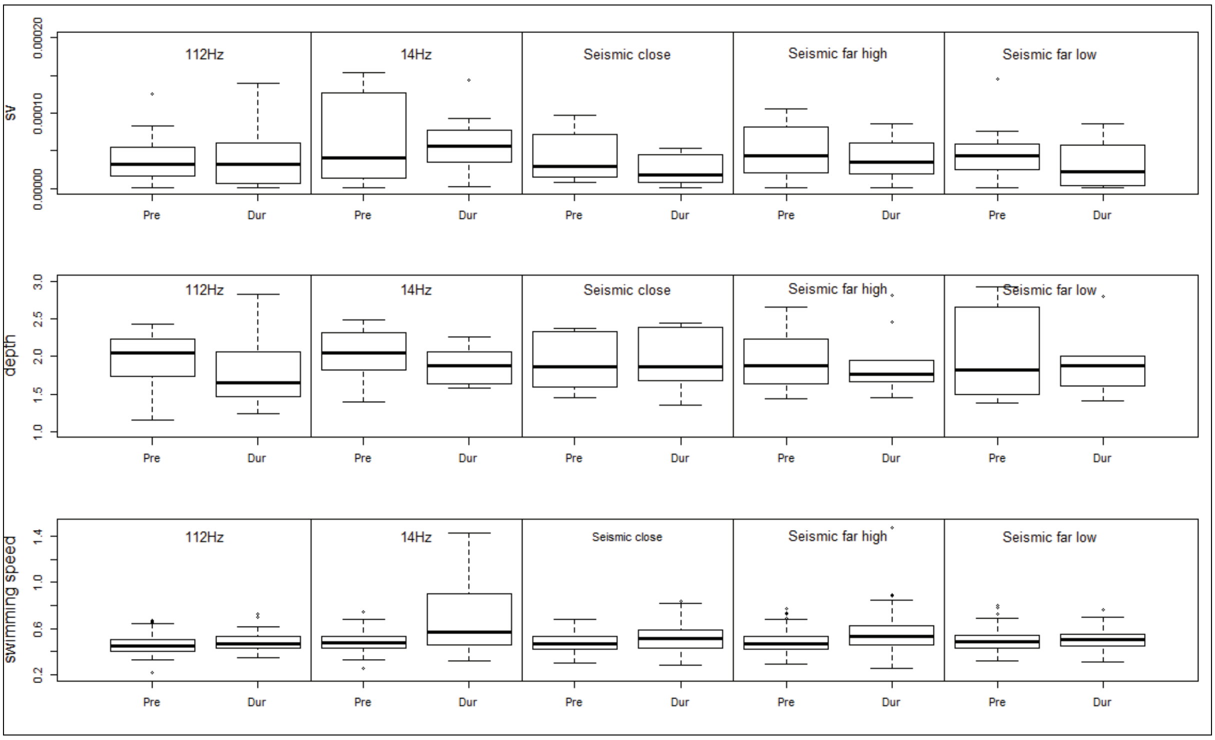

Figure 15. Boxplot for the Pre exposure and During Exposure phase for the three response variables sv (upper panel), depth (middle panel) and swimming speed (lower panel) for the 5 different treatments.

We used the same basic model as when looking at the Pre data, and used model selection to reduce the initial model. Data were log transformed to obtain homoscedasticy of the residuals (all variables). Using model selection we found the best general model to have all factors (batch, block and order) as fixed factors. The general model for all three variables were:

```
model2=lm(log(response.variable)~Block*binary.response+order+factor
(Treatment)*binary.response+factor(Batch)*binary.response
```

With the response variables being either depth, sv or swimming speed.

For *swimming speed*, the final model, after model selection, was

```
model2b=lm(log(swim.speed)~Block*binary.response+factor(Treatment)*b
inary.response+factor(Fish.batch)*binary.response)
```

This model showed that the 14 Hz treatment gave a significant effect ($p<0.001$); with an increase in swimming speed of ~$0.26 ms^{-1}$. Furthermore, the seismic far high treatment had a significant, but weaker ($p=0.04$) effect with an increase in swimming speed of ~$0.088 ms^{-1}$. Additionally, there was an effect of batch, with fish batch 3 standing out ($p=0.05$) with higher swimming speeds (increase of about $0.1\ ms^{-1}$) compared to batch 1.

For *depth*, the model selection removed both order and block, and the final model was:

```
model2c=lm(log(swim.speed)~factor(Treatment)*binary.response+factor(
Fish.batch)*binary.response)
```

The results indicates no significant change in the depth distribution ($p=0.45$) in response to the exposure of any treatment.

For *sv*, the model selection removed order, keeping block and batch, and the final model was:

```
model2b=lm(log(sv)~Block*binary.response+factor(Treatment)*binary.re
sponse+factor(Fish.batch)*binary.response)
```

The only significant effect was the pre data for fish batch as a factor, indicating that the mean *sv* was slightly different between the bathces. This is probably due to small differences of fish in each batch.

4.2 Video scoring

For all three scoring categories, the 14 Hz signal produced the highest scores (figure 18), and was the only exposure that received any score 3 (figure 19). Importantly, C-responses were only induced by the 14Hz stimulus (figure 19a). Changes in schooling behaviour were also mainly seen in response to the 14 Hz signal (figure 19c). S-responses (score 2 of category A: Startle behaviour) was also seen in response to seismic far high, as well as in one occasion each for the 112 Hz and seismic close (figure 19a). A typical reaction to the 14 Hz signal, involving both C-start and collective response is shown in figure 20.

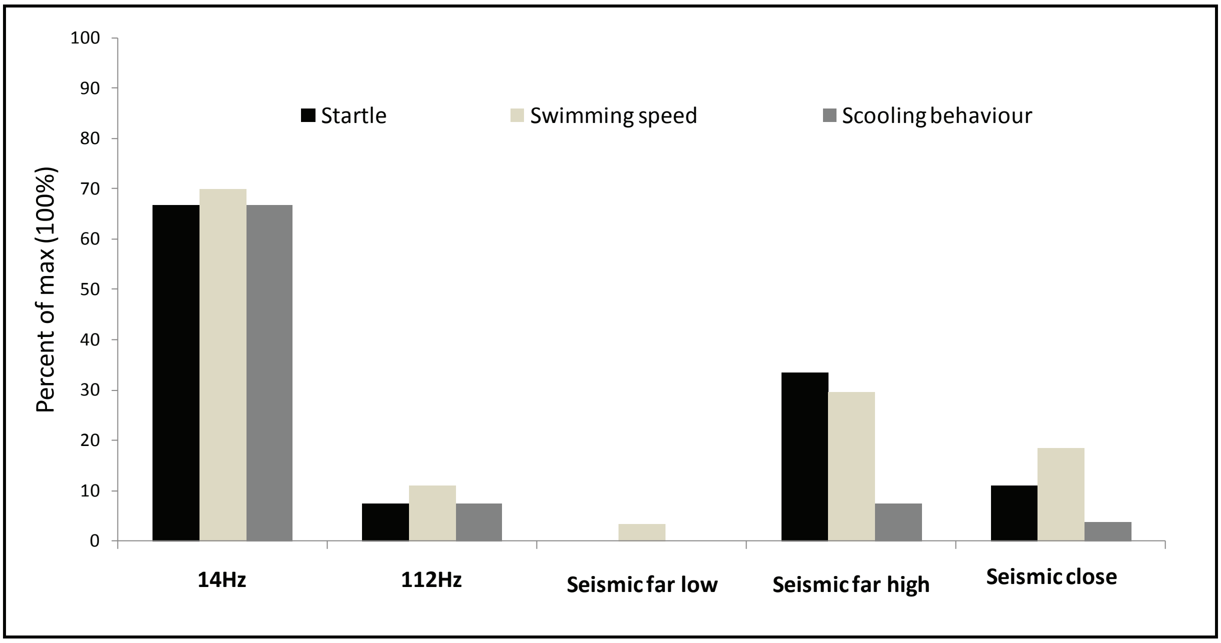

Figure 18. Results from video scoring. The figure shows the total summarized score within each category. Maximum (100 %) is score 3 in all exposure runs.

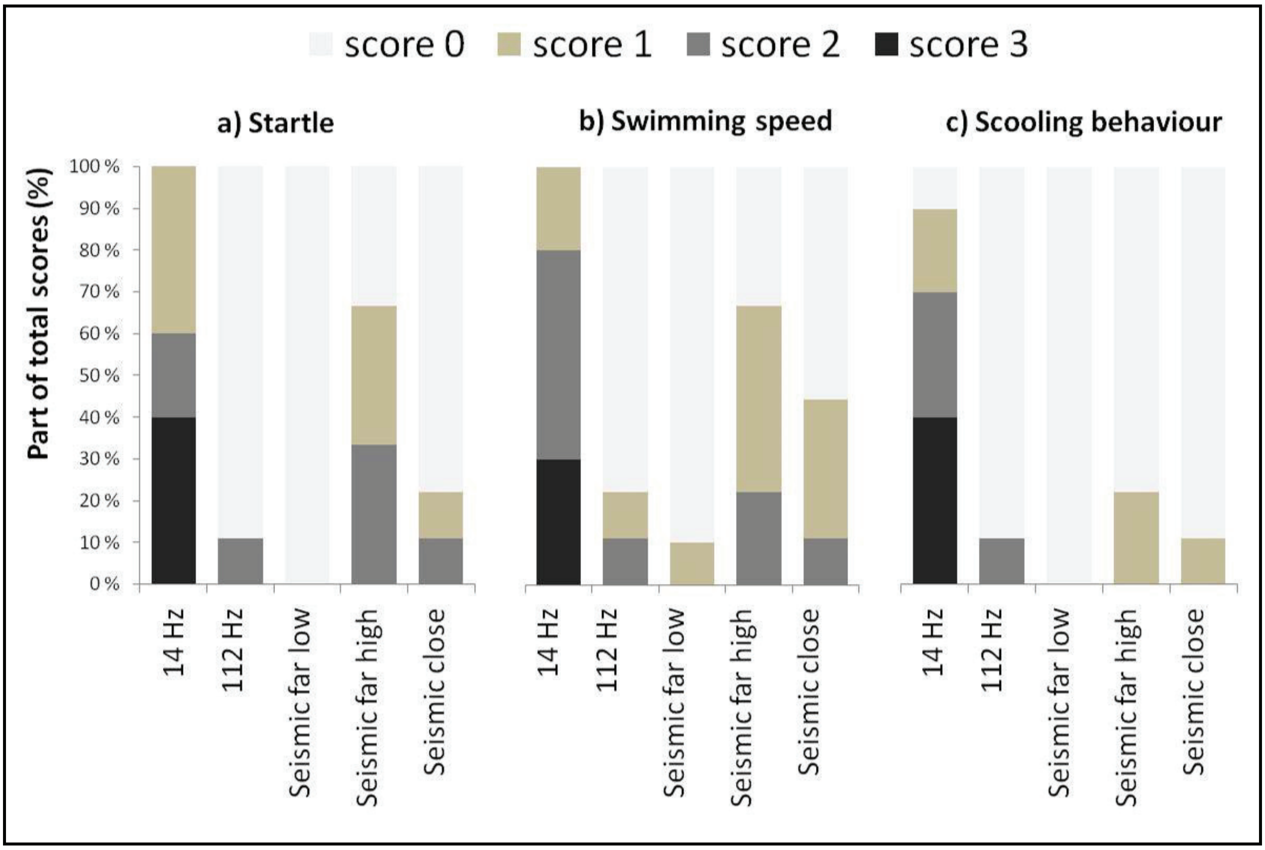

Figure 19. The contribution of each score value (0-3) as a fraction of the total for the categories a) startle responses, b) changes in swimming speed and c) changes in schooling behaviour/collective responses.

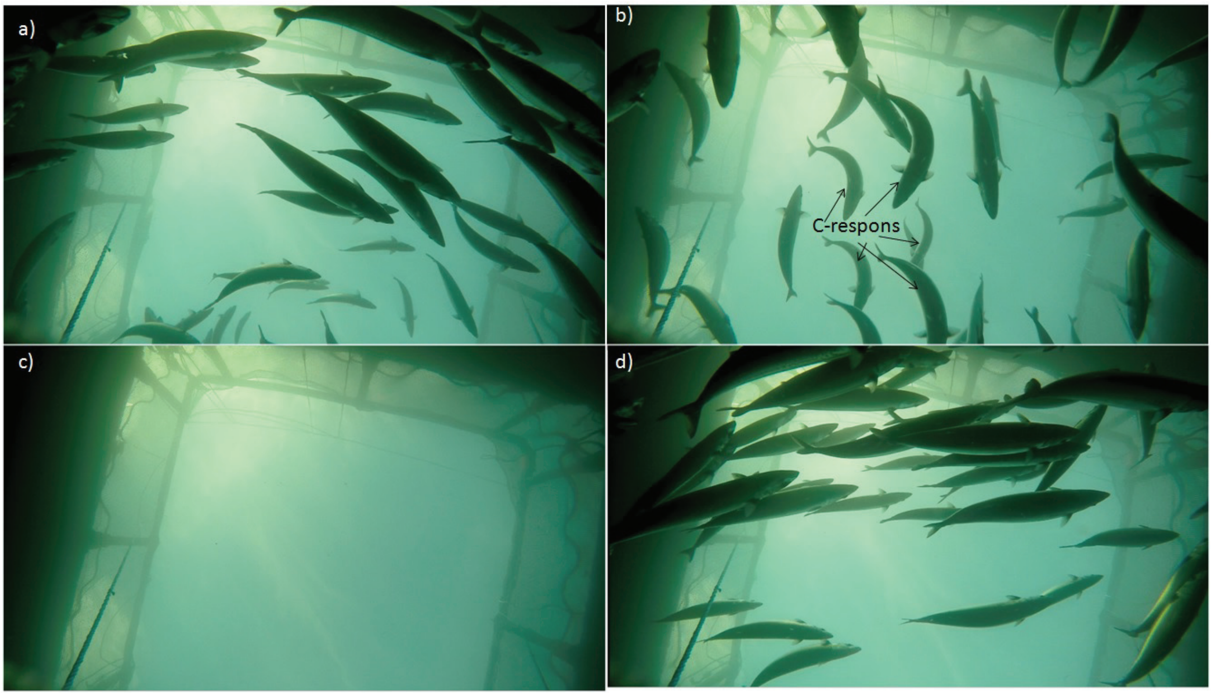

Figure 20. Example of reaction. a) 3 sec before exposure start b) during the exposure, showing the fish doing a C-startle response. c) 3 sec after ended exposure and d) 10 sec after ended exposure, showing fish swimming closer and with more coordinated movement than before exposure start.

Statistics of the scoring data were done by wilcox tests, comparing the different treatments. Here we do not have any baseline dataset to compare with, and are mainly focusing on the differences between the treatments, and this is a highly robust test for testing such differences.

For scoring category A, startle responses, 14 Hz had significantly higher scores than all the other treatments ($p<0.01$), and seismic far high had significantly higher score than seismic far low and 112 Hz ($p<0.05$) and almost significantly higher than seismic close ($p=0.08$).

Almost the same result was found for scoring category B, swimming speed; 14 Hz was significantly higher than all other treatments ($p<0.01$). Seismic far high was significantly higher than seismic far low ($p=0.013$).

For scoring category C, schooling behavior, 14 Hz was significantly higher than all other treatments ($p<0.01$), but none of them differed from each other.

5 Summary and discussion

The main purpose of this pilot study was to test and evaluate the experimental set up of studying behavioural responses to sound stimuli of mackerel in a net pen, and give recommendations for future studies.

Secondly, we proposed two scientific objectives, 1) testing whether mackerel respond more strongly to a) the peak pressure of a seismic signal, b) energy content, c) or different temporal structure simulating signal at different ranges. 2) Comparing responses to infrasound to that of a similar signal at higher frequency.

Below we have addressed these two objectives.

5. 1 Method evaluation and recommendations for future studies

Results from this project show that behavioural responses of mackerel can be well studied in net pens. The size of the batches was good, giving suitable space in the pen of the size used here, and the school was large enough for schooling behaviour to occur. However, if possible, the pen could be deeper, giving more room for a diving behaviour.

In this pilot study, we used a set of different monitoring systems to be able to evaluate which was the best to capture potential reactions. Video is a good tool to capture the essence of what is going on, but may be difficult and time consuming to quantitatively analyze.

Data from the echosounder did not show any statistically significant results. This may be due to the time window used (10 sec intervals) not being adequate, but a visual inspection of echograms indicate that no clear, consistent change was seen. If an echosounder had been our only "window" into the pen, we would probably not have been able to detect the strong reactions that the video captured. It is therefore more likely that an echosounder is not the most appropriate tool to measure the relatively brief reactions (startle responses) seen for mackerel, particularly in such shallow pens as used here, were the vertical movement anyway is highly limited. In the event of a future study, employing a similar sized net pen, video recordings could be prioritised as the main method of sampling data. In that case, a stereo camera set up would be desired to allow for three dimensional analysis of the fish behaviour.

Of the three seismic playbacks, treatment seismic far high came out as the ones giving the strongest responses. This treatment differed from the others in terms of SEL, indicating that higher SEL also may be important to induce a response. Due to the low frequency cutoff at about 50 Hz, the seismic signals did not include frequencies below this. The playback signal is therefore not truly representative of a real seismic signal, with frequency components well into the infrasound range. With the strong response seen towards the infrasound signal, such playbacks may not be recommended used in future studies. Rather a source having the capabilities to produce frequencies into the infrasound range should be used. The infrasound source also used here could be used, but as only producing a pure tone at 14 Hz, it is not fully representing a seismic signal either. The best will be to use a real seismic source, e.g. a small scale source as are used during routine operations and site surveys by the seismic industry.

For those blocks having three succeeding blocks in the same batch, there was some indication of lowered reactions in the third block. This may indicate some degree of habituation, or that the block was separated too close in time (1 h apart). Additionally, analysis of baseline swimming speed of the fish showed a decrease with increasing block for each batch (figure 15), indicating either a daily pattern, or that the fish may be stressed from the transfer, and slowly settling down from this throughout the day. It is therefore recommended that fish is transferred the evening before the experiment, thus having the night to calm down, and rather do two blocks with sufficient time between (> 1 h) than three blocks with ~1 h between. However, studying the habituation effect is in itself highly interesting, and conducting several consecutive blocks, one could use the fist block to study the intuitive response and this together with the following ones to look at habituation.

While planning this experiment, we were not sure we would be able to measure particle acceleration. The experimental design with the 5 treatments was therefore planned to investigate for the different pressure components that can be measured with a hydrophone. However, the measurements of particle acceleration were done successfully, and added a key dimension to the results. In particular for mackerel, not being sensitive to the pressure component, but merely the particle acceleration, it will be of high importance to measure this in future studies.

Shortly, recommendations for methodology in future studies are therefore:

- Use a good and stable video camera system, preferably a stereo camera
- Use of a source that enable transmitting the full frequency spectrum
- Transfer fish to the measurement pen the evening before the experiment start
- Carefully evaluating block design with respect to number of blocks and time between blocks within the same batch, bearing in mind if we wish to evaluate initial response and/or habituation.
- For a similar sized net pen (5x5x5 m) similar sized batches (2-300 fish) should be used.
- If possible, a deeper net pen should be used to better allow diving behaviour.
- Measure particle acceleration

5. 2 Scientific results

By far the strongest reaction was seen in response to the 14 Hz signal, including C-start, increased swimming speed, increase in synchrony between individuals and occasionally change in vertical distribution. The 112 Hz signal were playback of a recording of the 14 Hz signal played back 8 times faster, hence the higher frequency For objective 2); comparing responses to infrasound to that of a similar signal of higher frequency, it is therefore clear that the infrasound triggered far stronger reactions. It is therefore tempting to conclude that infrasound is important in triggering the strong reactions seen. However, that we were able to also measure particle acceleration adds another dimension to this, with the 14 Hz signal being about an order of magnitude stronger than the 112 Hz signal (0.03 and 0.007 ms^{-2}, respectively). Thus, we cannot say for sure whether it is the high particle acceleration or the presence of infrasound that triggered the responses seen in response to the 14 Hz signal.

For objective 1); testing whether mackerel respond more strongly to the peak pressure, energy content or temporal structure of the signal, the seismic far high signal, gave strongest responses of the three seismic playback signals. This indicate that total energy content may be most important of these sound characteristics.

To summarize the main findings of this study, we have documented strong responses in terms of C-start, increased swimming speed and schooling dynamics to intrasound with particle acceleration at 0.03 ms^{-2}. Weak and moderate responses were documented to signals of higher frequency content (50 - 300 Hz) at merely 1/10 of the acceleration levels for the infrasound signal, indicates that stronger reactions might occur at greater acceleration levels also for signals of higher frequency. Results also indicate that total energy content of a sound signal may be important to induce behavioural reactions of fish.

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