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Sivle Lise Doksæter; Tonje Nesse Forland, Rune Roland Hansen, Mathias Andersson, Endre Grimsbø, Markus Linne, Hans Erik Karlsen

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Adferdseffekter av seiskmikk på makrell (Scomber scombrus)

Behavioural effects of seismic dose escalation exposure on captive mackerel (Scomber scombrus)

Authors:
Sivle Lise Doksæter; Tonje Nesse Forland, Rune Roland Hansen, Mathias Andersson, Endre Grimsbø, Markus Linne, Hans Erik Karlsen

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Summary (Norwegian):
Petroleum og fiskeri er to av de viktigste næringene i Norge, og målsetningn er en forvaltning som sikrer god sameksisens mellom de to næringene. Makrell er en av Norges viktigste pelagiske fiskeriressurser. Makrellen har god hørsel i de frekvensområder seismikken opererer i, og kan derfor potensielt påvirkes av slik kraftig lyd, f.eks. ved å svømme vekk eller dykke. Målsetningen med dette prosjektet var å undersøke slike adferdsresponser, og å evaluere hvordan de potensielt kan påvirke dens fangbarhet. Villfanget makrell i merd ble studert ved hjelp av ekkolodd og videoovervåkning I merden mens den ble eksponert or gradvis økende lyd fra en luftkanon som ble tauet etter et fartøy som kjørte mot merden. Lydnivå inne i merden i form av trykk og partikkelakslerasjon ble målt med hydrofoner og en partikkelakslerasjons-sensor. Fiskens adferd be analysert i form av svømmehastighet, vertikal fordeling i merden, samt gruppedynamikk og stimadferd. Vi ønsket å gjøre forsøket som et dose-eskalerings forsøk for å kunne identifisere ved hvilke lydnivåer ulike adfersresponser inntraff. Imidlertid fant vi ikke noen tydelige adferdsresponser i respons til seismikklyden, slik at noen slik terskel for respons ikke kan fastsettes basert på våre resultater. I tillegg til adferdsreaksjon hos makrell, undersøkte vi også tilsvarende hos laks og ørret på tre nærliggende oppdrettsanlegg, hvor adferd ble observert ved hjelp av videoovervåkning i merdene. Ingen endring i adferd hos oppdrettsfisk kunne verifiseres

Summary (English):
Petroleum and fisheries are two of the most important industries in Norway, and the goal for management is sustainable coexistence for both. Mackerel is an important pelagic fishery resource, and mackerel can very well detect the seismic sound signals. The aim of this project was to investigate the behavioural responses of mackerel to seismic signals, and to evaluate potential responses in terms of affecting the fishery. Wild captured mackerel in a net pen was exposed to escalating seismic signals from an approaching source vessel, while behaviour was constantly monitored with video and echosounder, as well as the sound pressure level and particle motion level recorded with hydrophone and particle motion sensor, respectively. Fish behavior was analyzed in terms of swimming speed, vertical distribution, schooling and group dynamic. We aimed at conducting a dose escalation to identify the sound level at which a response is initiated. No clear responses were identified in response to the sound exposure. In addition, behavioural responses of farmed salmon and rainbow trout was monitored by video surveillance at three close-by aquaculture farms to avoid any potential harmful effects on the farmed fish. However, no behavioural responses in terms of swimming dynamic, swimming speed and collective behavior were observed from these videos

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Emneord (norsk):
1. **Makrell, seismikk, adferdseffekter**

Subject heading (English):
1. **Mackerel, seismic, behavioural responses**

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Content

1 Introduction

Petroleum and fisheries are two of the most important industries in Norway, and the goal for management is sustainable coexistence for both. There has been a repeated claim from fishermen that seismic activities in vicinity of fishing grounds of mackerel have caused the fish to abandon the area, resulting in lower catches. How seismic sound affects fish and how that can lead to reduced catch rates are thus key questions to be addressed for a constructive coexistence between these industries. It is of special interest to obtain improved knowledge on thresholds for behavioural disturbance, allowing improved estimates of possible reaction distances. Behavioural reactions of fish to sound are complex and often context dependent (DeRobertis & Handegard, 2012). Assessment of possible effect on fishery success introduces further complexity as it also includes variables like, e.g. fishing gear, noise impact of fishing vessel etc. Fish can show a variety of reactions to sound, ranging from abrupt and transient startle behaviour with very short duration to somehow longer behavioural reactions like diving, increased swimming activity and changes in schooling behaviour and structure (e.g. Slabberkoorn et al. 2010; Hawkins & Popper, 2017). Mackerel is a pelagic fish without a swimbladder. The lack of swimbladder reduces the hearing sensitivity in the upper frequency bands compared to, e.g. herring, but not in the main frequency range where seismic air-guns operates, with a recent study showing marcel to hear in the frequency range 5-250 Hz, with best hearing in the 40-160Hz band (Hansen & Karlsen, 2016). It has also been demonstrated that mackerel reacts to the exposures in these frequency ranges by recent experiments in net pens conducted by IMR and UiO and funded by Statoil. The experiments (reported in Sivle et al. 2016) showed:

- Mackerel show startle response, diving and increased collective behaviour when exposed to infrasound (14Hz) in net pen experiments.
- Behavioural responses seem to depend on frequency and/or level of particle acceleration, with low frequency and high particle acceleration inducing stronger responses than the higher frequency and lower particle acceleration.
- The sound exposure level seems to be more important than peak pressure levels.

In this current study, we will expose mackerel to a real seismic air gun and investigate the nature of the behavioural response; with emphasis on possible effects on fishery. This includes documenting the type of responses, the duration of responses aas well as trying to identify the specific level of onset of a response. Due to important mackerel fishery being conducted with baited hooks, we were also interested in studying if their willingness to feed changed during exposure. We conducted a field experiment in November 2016, with mackerel in net pens and their behaviour monitored during exposure to a seismic air gun towed from a vessel approaching from a distance. The aims of this experiment was to

a) Conduct a dose-escalation experiment

b) Identify received sound pressure levels (SPL) and sound exposure levels (SEL) at which certain behavioural reactions are triggered.

c) Investigate changes in the willingness to take food pellets with and without exposure to seismic air guns.

d) Evaluate how potential changes in behaviour can affect fishery and catchability of mackerel.

2 Materials and Methods

2.1 Experimental location and logistic components

The experiment was conducted in Bjørnafjorden in western Norway, with fish being held at the IMR research facility at Austevoll, in a sheltered bay (Figure 1). During experiments, different batches of fish were towed sequentially to the experimental location in the main fjord. The experimental location was a closed fish farm, but with available moorings for both the net pen and observation vessel.

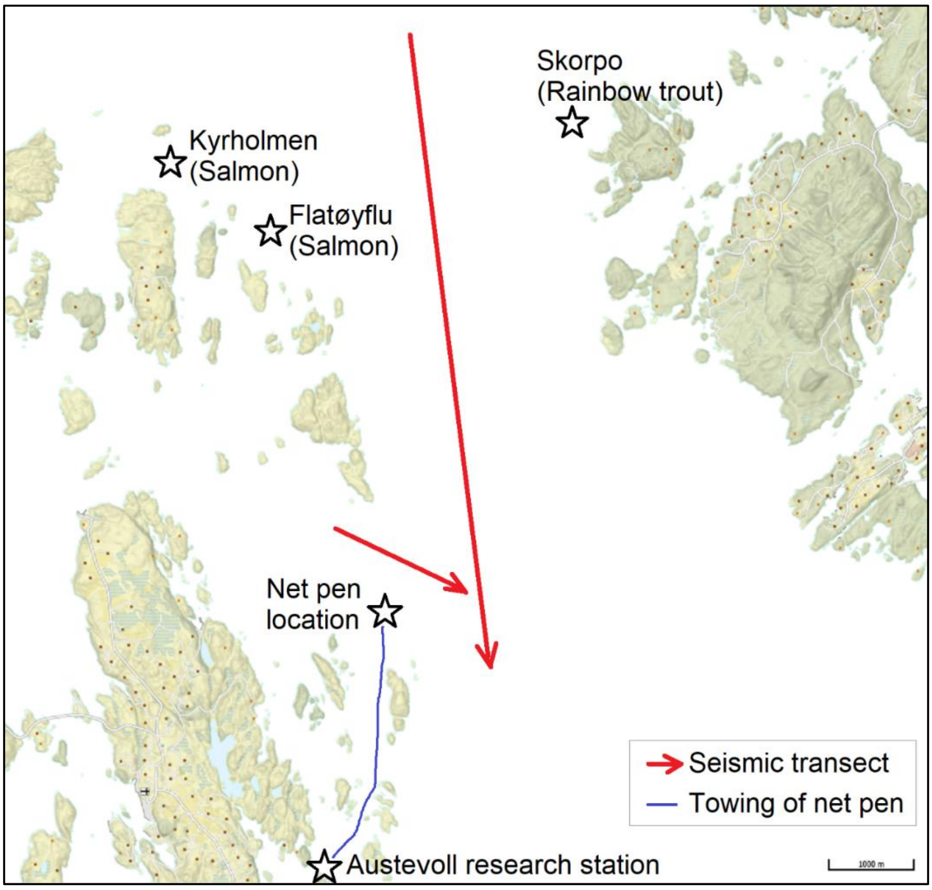

Figure 1. *Map of operation area. Mackerel was held at Austevoll research facility, and transferred to smaller net pens that was towed to the experimental position (net pen location). The position of the two transects are indicated; seismic dose escalation (long arrow) and seismic short (short arrow). Behaviour of farmed fish were done at two locations housing two different fish species; salmon in Flatøyflu and Kyrholmen, and rainbow trout in Skorpo. Right corner show the location of Austevoll in west Norway.*

Fish were held at Austevoll research facility in large net pens (12x12 m wide, 10 m deep). Two or more days before each experiment, a batch of fish (approximately 200 individuals) were transferred to a smaller (5x5 m wide, 10 m deep) pen (Figure 2). The pen was then towed by a small boat to the experimental location, located outside of the islands surrounding the stations. were it was moored between two buoys.

This was done to prevent exposing other fish at the station to the seismic sound as well as to be able to not expose all mackerel at once, but to conduct exposure with several batches of fish thus to increase the sample size of exposure to novel stimuli.

Figure 2. *Net pen used in the experiment (left) and during towing (right). Experienced personnel from Austevoll research facility was observing fish during towing aiming to avoid the fish getting stressed.*

The experimental location and seismic transect was located such that the research station would be as best as possible sheltered by islands for the sound from the seismic to propagate in to the research station. Towing was always done the day prior to the experiment, to let the fish acclimatize over night before the experiment started. Towing was done at low speed to minimize stress on the fish.

Three vessels were used in the operation, the two IMR research vessels "Hans Brattstrøm", hereafter Brattstrøm, and "Håkon Mosby", herafter Mosby, in addition a small private vessel (a Targa 27), hereafter the Targa, that was rented for the towing operation (Figure 3).

Figure 3. *Vessels used. a) Håkon Mosby was used as source vessel, housing the air gun. All scientific personnel was also housed in this vessel. b) Hans Brattstrøm, used as surveillance vessel. All monitoring equipment was housed and set out in the net pen from this vessel. c) a Targa-27 was used to tow the net pens between the experimental location and Austevoll research facility*

Brattstrøm was used as observation vessel, housing all the observation equipment, and with the net pen being moored close. Mosby was used as source vessel, housing the air gun, conducting the seismic exposure of the fish in the net pen. Seismic exposure was conducted by using a Bolt 1900 air gun with a volume of 90 cubic inch, with source level of 1.8 bar/ 223 dB re 1µpa @ 1 m. The Targa towed the net pen back and forth between the research facility and the experimental location.

2.2 Experimental set up

The evening before an exposure experiment, a net pen with about 200 mackerel in were towed and moored to the buoys at the experimental location. The fish were left overnight to calm down after any potential towing stress. In the morning, Brattstrøm came and positioned itself at the side of the pen, and all the monitoring equipment were placed into the pen. When all monitoring equipment was up and running, the fish had some time to calm down before logging of baseline data started. Then Mosby positioned itself for the first exposure run. Simultaneously, behaviour of farmed fish at two locations was monitored b during exposure to prevent any stress on farmed fish. Transects and position of the net pen and fish farms are shown in Figure 1. Each of the operational days as many as possible exposures were done to maximize the dataset. Four different types of runs were conducted:

Seismic dose escalation – Transect starting 4.5 nautical miles (nmi) from the net pen, continuing about 0.5 nmi after passing it. The closest point of approach (CPA) between the source vessel and net pen was about 300 m. The intention was for the fish in the pen to experience a gradual increase in sound level, thus to be able to pin point at what received level a certain reaction occurred. This type of exposure will also represent a situation of a real operation with a cyclic increase and decrease in level as the source vessel move around during a survey.

Seismic short – a shorter transect, starting about 900 m away from the net pen, mainly to try to get an even closer closest point of approach (CPA) than for the dose escalation transect. This transect approached the net pen from a different angle, but could not be as long due to navigation in an area with several small islands. This transect therefore had a higher level of sound for the first pulses, hence a less soft start than for the seismic dose escalation.

Seismic still close – this was more of an “ad hoc” experiment conducted the last day, as to try getting the source even closer to the pen, thus increasing the sound level inside the pen. The source vessel was then positioned approximately 90 m from the pen, not moving, shooting 40 seismic shoots.

Control no seismic – passage of the source vessel along both transects described above, but without any seismic, to be able to distinguish any reaction to the vessel itself from that of the seismic sound.

2.3 Data collection

Data on behaviour and sound was collected by hydrophones, videocameras, and echosounder (Figure 4).

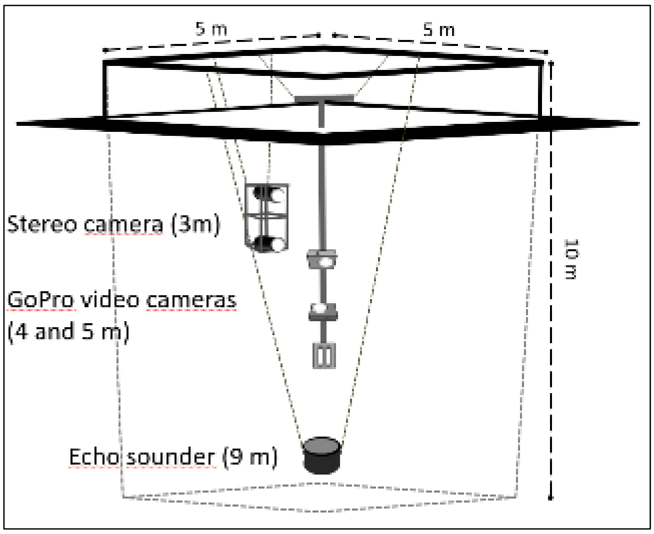

Figure 4. *Setup of equipment for behaviour monitoring. The pen was 10 m deep. Echosounder was placed in the bottom of the pen, looking upward. Stereo camera in a rig looking horizontally into the pen, and go pro cameras in a different rig placed deeper.*

2.3.1 Sound pressure

Two Brüel & Kjær hydrophones, B&K 8106, were positioned at different depths and horizontal positions inside the net pen (Figure 5). The hydrophones were hanging from their cables at depths of about 3 and 5 meters. The hydrophones detected the sound pressure inside the net pen. Both vessel noise and seismic signals were detected. The hydrophones were used in all blocks of the experiment. Before and after the experiments, the hydrophones were calibrated with a B & K 4229 hydrophone calibrator with a B&K WA 0658 coupler.

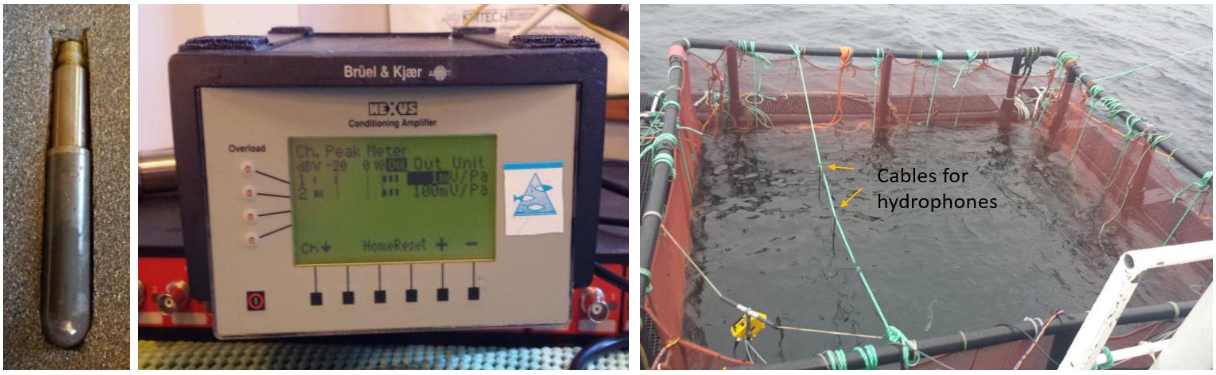

Figure 5. *Hydrophone (left) and amplifier (middle) and positioning of hydrophones in net pen (right). The depths for the two hydrophones were 3 and 5 m.*

2.3.2 Particle motion

The Particle Motion sensor (PM-sensor) used in this project is custom build system based on the design described in Sigray & Andersson (2011). The difference is that the sensor used herein is autonomous and the sphere is smaller. The sensors platform consists of two water-proof electronic units, one containing rechargeable lithium batteries and the other the data acquisitions system. The nearly neutrally buoyant sphere has a diameter of 0.06 m and is kept suspended 0.3 m above the unit (Figure 6, left). Inside the sphere is a PCB Piezotronics, model 356B18, 3-axis accelerometer mounted, with a flat sensitivity in the frequency interval (+/-5%) of 0.5 Hz – 5 kHz. The sensitivity of the accelerometer is 1 V/g, g being the gravitational constant of ~9.82 m/s^2. The sensors noise floor at 10 Hz is 4 µg/Hz$^{1/2}$= 32 dB re 1 µm/s^2 and at 100 Hz is 1.2 µg/Hz$^{1/2}$= 22 dB re 1 µm/s^2. The sampling frequency is 14400 Hz and the resolution of the Analog-to-Digital converter is 24 bit. The recorded data is stored on a 32 Gb SD-card. The sensor has a hydrophone (Cetacean C55RS, sensitivity -180 dB re 1V/µPa) connected to the data acquisition system which allows for synchronized recording of both pressure and particle motion. The PM-sensor was suspended at 5 m depth from the stern of Brattstrøm (Figure 6, right), approximately 5 m from the the Brüel & Kjær hydrophones inside the net pen.

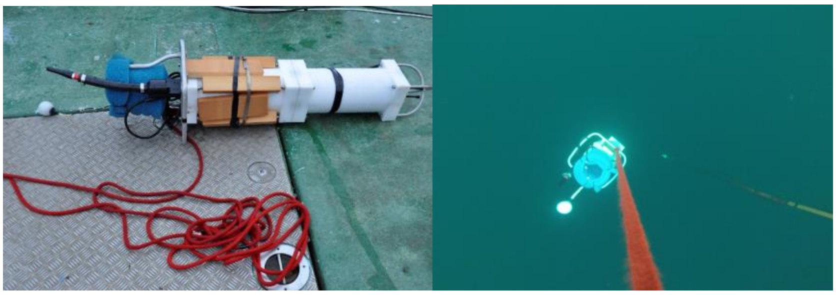

Figure 6: *FOIs particle motion sensor on deck on Brattström (left) and in the water (right). The grey orb contains the three-axis accelerometer and the hydrophone is the black rod to the left in both pictures.*

2.3.3 Echosounder

An echosounder (120 kHz split-beam echosunder, Simrad EK 60, Kongsberg Maritime AS, Horten, Norway) was placed close to the bottom of the net pen. The echosounder was mounted in a specially made holder which was hanging from two ropes. A weight ensured that the transducer was facing upwards.

2.3.4 Video

High-resolution video recordings (1080p 25 fps) were captured with two GoPro (Hero4 black) cameras placed inside the pen, documenting the behavior of mackerel schools before, during and after exposure to air gun sounds. The cameras were linked to pc-screens on the observation vessel via video cables, which enabled live monitoring of the fish. Fixed to a custom made camera pole, the GoPros were suspended from the pen railing at 4 and 5 m depth and directed approximately 120 degrees down and 70 degrees up, respectively, relative to the surface. With this arrangement, we managed to cover the major and key areas inside the pen (Figure 7). Some adjustments of the cameras and pole were necessary on day-to-day basis, depending on the shape and angles of the suspended net, which was highly influenced by the prevailing current velocity and direction.

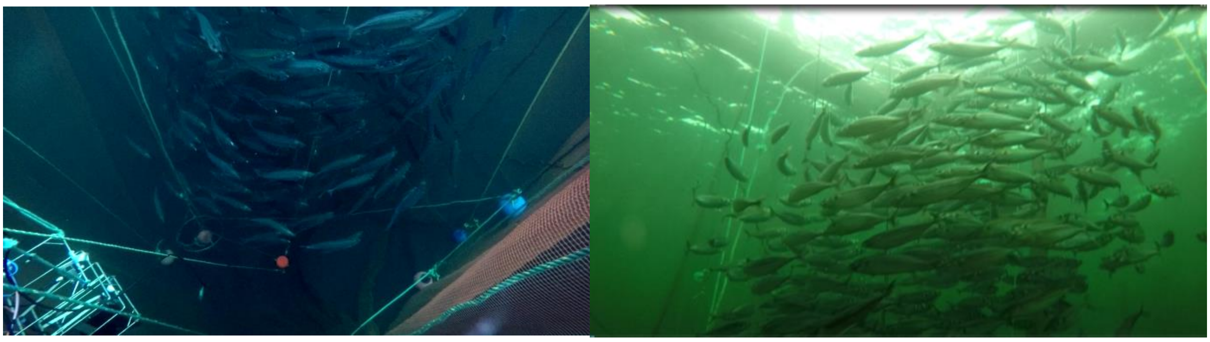

Figure 7. *Example of video images from go pro camera placed at 4 m (left) and 5 m (rigth) depth, pointing 120 degrees down and 70 degrees up, respectively.*

2.3.5 CTD

CTD is an instrument which can detect conductivity, temperature and hydrostatic pressure in sea water. Depth is estimated from measurements of hydrostatic pressure, salinity is derived from the conductivity and speed of sound can be found based on hydrostatic pressure, salinity and temperature. CTD-measurements were made once a day from the 21. to the 25. of November (Figure 8).

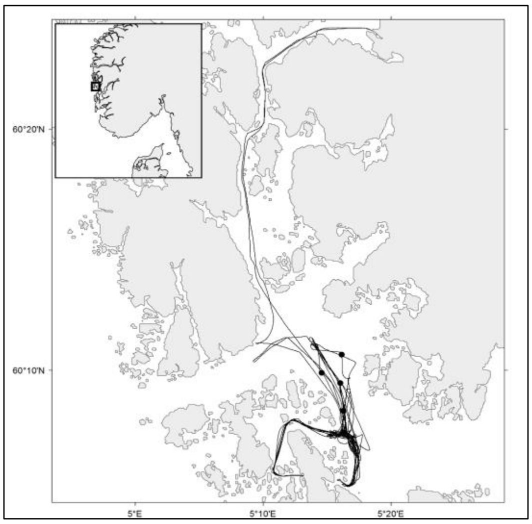

Figure 8. *Map showing location of CTD casts as black filled circles, overlaying the track of «Håkon Mosby»*

2.3.6 Measurement of feeding activity

One of the objectives of this study was to identify changes in appetite/willingness to feed during exposure to seismic air gun compared to without such disturbance. During our previous experiment, in

October 2015, mackerel had a very high appetite on food pellets, and responded instantly by taking these when offered. We therefore planned to design a good way of feeding a limited number of pellets to the fish, by carefully introducing them into the water just next to a vertically aligned sets of go pro cameras to identify the time taken before the pellets released was eaten. To test this, and to refine a design the best way of measuring and testing this, we spent a week at Austevoll to refine methods and design the best set up for measuring feeding and find the best way of quantifying feeding. However, during this week, it became clear that the fish was not very interested to take the food pellets. We tried different approaches, but concluded that with the limited appetite as for baseline, it was not possible to find any good way of measuring this. When the fish was not feeding in the first place, it will not be possible to determine if their feeding activity decrease during exposure.

2.4 Data analyses

2.4.1 Analyses of acoustic environment (sound pressure and particle acceleration)

The analyses of the acoustic measurements consist of Energy Spectral Density calculations, Zero to Peak calculations and the time-integrated squared sound particle acceleration (AEL) and Sound Exposure Levels (SEL) calculations. Sequences of 1 s around the seismic pulses were selected and analysed, with the pulse placed early in the time frame. This was done to capture lower frequency oscillations coming in the aftermath of the main pulse. See Figure 9 for examples of graphs showing time series from one pulse.

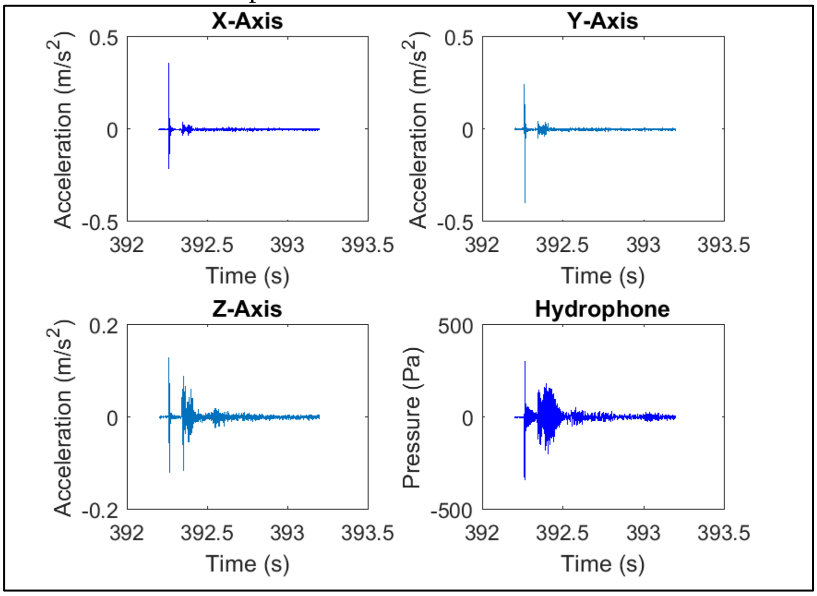

Figure 9. *Time series from a seismic airgun measured both with the PM-sensor and the hydrophone. The X-, Y- and Z-axis are the accelerations measured with the PM-sensors three different axis. The subplot with the title "Hydrophone" shows data measured with the Cetacean C55RS hydrophone. This particular pulse is from around CPA.*

The platform outside the net pen consists both of a particle motion sensor (PM-sensor) and a hydrophone, calculations has been done both of the particle motion and (sound) pressure variations in the medium. Since the acceleration has three directions these has been combined together giving a total acceleration in these calculations. For more easy comparison with earlier studies, the PM data are presented both in dB re 1 $\mu m/s^2$ and m/s^2 for zero-to-peak and time-integrated squared sound particle acceleration dB re 1 $\mu m^2/s^3$ and m^2/s^3.

The Energy Spectral Density, ESD, calculations are very similar to standard Power Spectral Density Calculations, PSD. The received signals from the seismic gun are short pulses of energy in the time domain and therefore the ESD calculations are more suitable. The PSD calculations are generally used for continuous signals. In Figure , there are ESD spectra presented for the pulse presented in Figure.

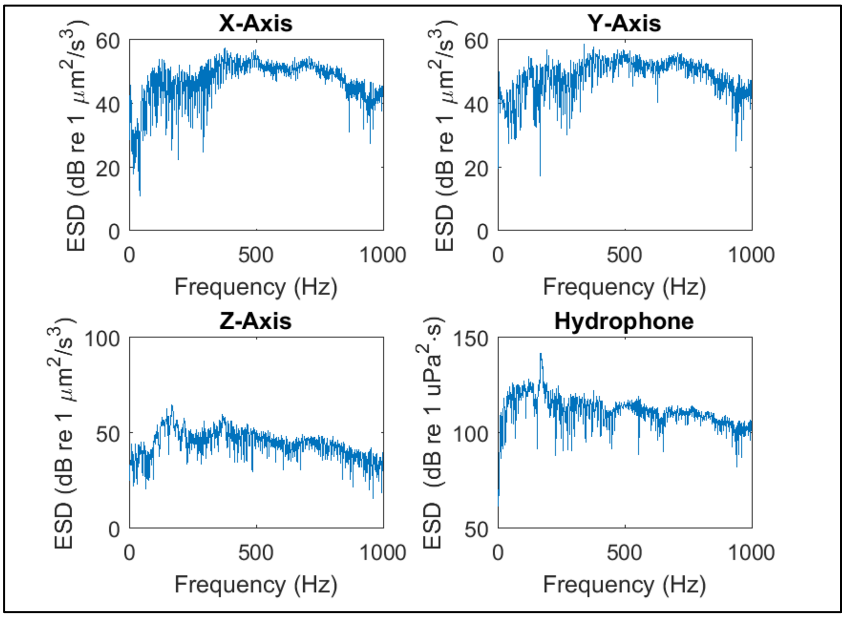

Figure 10. *The Energy Spectral Density (ESD) for the airgun pulse presented in Figure 9.*

The Zero to Peak (02P) calculations has been extracted from the time series of the measurements. It is simply the maximum value (Positive or negative) in the analysed time series.

The time-integrated squared sound particle acceleration (AEL) and Sound Exposure Levels (SEL) are derived from the ESD calculations and is the integral of the square of the magnitude over a specified time interval or event, for a specified frequency range. First, the energy is integrated in each pulse in the frequency range of 5 to 400 Hz. This frequency range was chosen after some discussion within the project group and is related to the estimated hearing range of mackerel ("hearing filter"). Second, the integrated energies are summed up for all the pulses (e.g. 40 pulses in Block 8 run 1) giving a total dose of energy as a function of pulse number.

A number of airgun pulses has been selected to show the variation frequency content and received level. For Block 6, pulse one is one of the first airgun pulse registered at roundly 7000 m distance, pulse 188 is at a distance of about 3500 m and pulse 300 is at CPA, about 330 m.

The ESD analysis is done in the frequency range of 5 to 1000 Hz to show the maximum bandwidth of the signal that the platform can deliver. Above 1000 Hz, the PM sensor experiences some resonances, making the data unreliable. These data are presented in 1/3 octave band format.

In addition to the particle motion and hydrophone platform which was placed just outside the net pen, sound pressure was also measured with two B&K hydrophones inside the net pen at 5 m (hydrophone 1) and 3 m (hydrophone 2) depths. The analysis is done in the same way as described above, and the results from the 3 different hydrophones corresponds well to each other. For the hydrophones inside the net pen the results are studied both with the "hearing filter" (bandpass filter from 5-400 Hz), and also unfiltered. This gives an idea of how much of the signal the fish might not be able to detect. Figure 11 shows an example of the pulse before and after the filter is applied. It is interesting that the "rumble" after the first peak seem to be more important than the first peak when the "fish hearing filter" is applied.

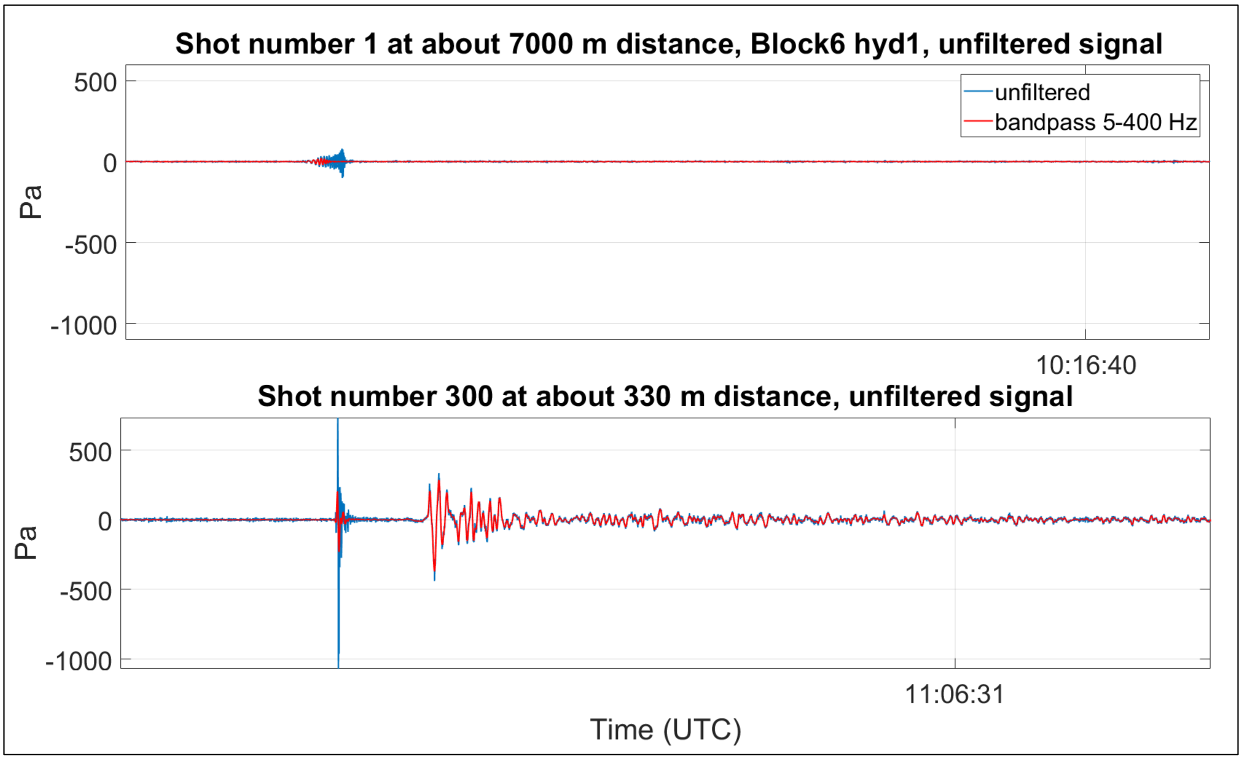

Figure 11. *Compare unfiltered signal (blue) to the same signal filtered with a 5-400 Hz bandpass filter (red curve). The figure shows 1 second long sequences of the signals. The upper plot shows the first pulse from block 6 at a distance of about 7000 m. The lower plot shows the seismic pulse at the closest distance between air gun and hydrophone of about 330 m. The red curve indicates what the fish can hear.*

2.4.2 Analyses of behaviour

Video data was used to measure swimming speed as well as for expert scoring of behaviour. Echosounder data was used to investigate the vertical distribution of the fish in the net pen.

Swimming speeds of individual fish were measured by tracking their movements manually, frame by frame, using the open source tracking software, ImageJ. We selected video recordings specifically where the school of mackerel predominantly swam in a carousel fashion, and was within the centre view of the upwards-pointing GoPro camera. From videos comprising Block 6 (run 1), Block 7 (run 1) and Block 8 (run 1), we extracted 3 s duration sequences of still images with respective time stamps overlaid. Sequences were sampled at 5-minute intervals from the videos, starting 10 min before and ending 10 min after air gun exposures. Additional sequences were extracted for key periods during the sound exposure; i.e. the initial air gun pulses and CPA. Sequences during exposure were extracted so that the initial frame was precisely 5 s after onset of an air gun pulse, to ensure comparability among this subset of data. Additionally, we picked out a bulk of 2 s duration image sequences starting at the onset of the respective air gun pulses, in order to look for potential short-term (< 2 s duration) changes in swimming speeds; e.g. startle responses.

Tracking of individual fish was standardized according to a set of criteria. Fish considered "trackable" had to confine to the centre of the image, in order to avoid lens distortion effects. They also had to be fully stretched out in the initial frame, with its tail and nose visible to enable measurement of body length. Tracking was done by marking the positions of the nose and tail in the initial frame (for body length), and the nose traced in four succeeding frames, of 0.08 s time steps (Figure 12). The mean swimming speed of each fish was calculated (in pixels) from the three distances over which it had moved during the track. Lastly, a conversion from pixel units to cm was made on the assumption that all fish adhered to the mean fish length of 41.6 cm, as measured in a sample of experimental fish.

Figure 12. *Movements of individual mackerel in the software ImageJ.*

Expert scoring of videos was done by two behavioural experts together, based on predetermined scoring criteria within three categories (Table 1).

Table 1: *Overview of categories used for videoscoring*

	Score	Description
Category A : Coordination of individuals within school	**Score**	**Description**
	1	Low coordination, less than 50% of fish have same directionality
	2	Medium coordination, 50-90 % of fish have same directionality
	3	High coordination, more than 90% of fish have same directionality
Category B : Swimming speed of school	**Score**	**Description**
	1	Calm swimming
	2	Fast swimming
	3	Sprint
Category C : Behavioural mode	**Score**	**Description**
	1	Schooling/carousel; swimming around in circle
	2	Seaching: swimming around in pen, vertically or horizontally. If fish had not been restricted by pen walls, may have been avoidance.
	3	Stationary schooling, the school of fish is relatively stable in the same position in the pen, swimming just to adjust for currents and to keep its position.

A score in each category was given every 10 second. Scoring of videos always started 10 minutes prior to the first seismic pulse and lasted until 10 minutes after last pulse. A screendump for each change in behaviour was made to ensure consistency in scoring as well as to better document any change. Figure 13 show examples of screendumps for each scoring type.

Figure 13. *Examples of the different categories outlined in Table 1. Upper panel is school coordination for score 2 and 3, as score 1 was never used. Middle panel show school speed, however speed is difficult to fully capture in a picture. Lower panel show the three different mode types.*

The vertical position and spread of the fish was studied from the echosounder data, by measuring the depth and spread within the echosounder beam. All reflections seen on the echosounder was assumed to be mackerel. It was necessary to remove some strong reflections (possibly from one hydrophone) that disturbed the analysis. It was also necessary to separate the water surface from the fish layer. This was challenging for some of the data where reflections from the fish and from the rough water surface partly overlapped. The separation between fish and surface was done manually by using the software LSSS (Korneliussen et al. 2016). Reflections closer than 1 m from the echosounder was disregarded since they were in the nearfield. For some cases where there were two layers of fish, the upper layer of fish was not included in the analysis since it was difficult to separate it from the surface, and in order to make the measurements more comparable to each other. An example of how the data of interest was selected from the echogram for Block 6 is shown in Figure 14. The data of interest is seen between the manually drawn lines marked with “Analyzed layer”. The rest of the dataset is defined as zero. The output of the echosounder data is the volume backscattering coefficient, sv, which is the sum of the backscattering cross section of each target, divided by the volume element. The unit of sv is m^2/m^3 =m^-1 (i.e. scaled to 1 m^3). sv is given as dB re 1 m, so the data must be converted to linear scale before the analysis.

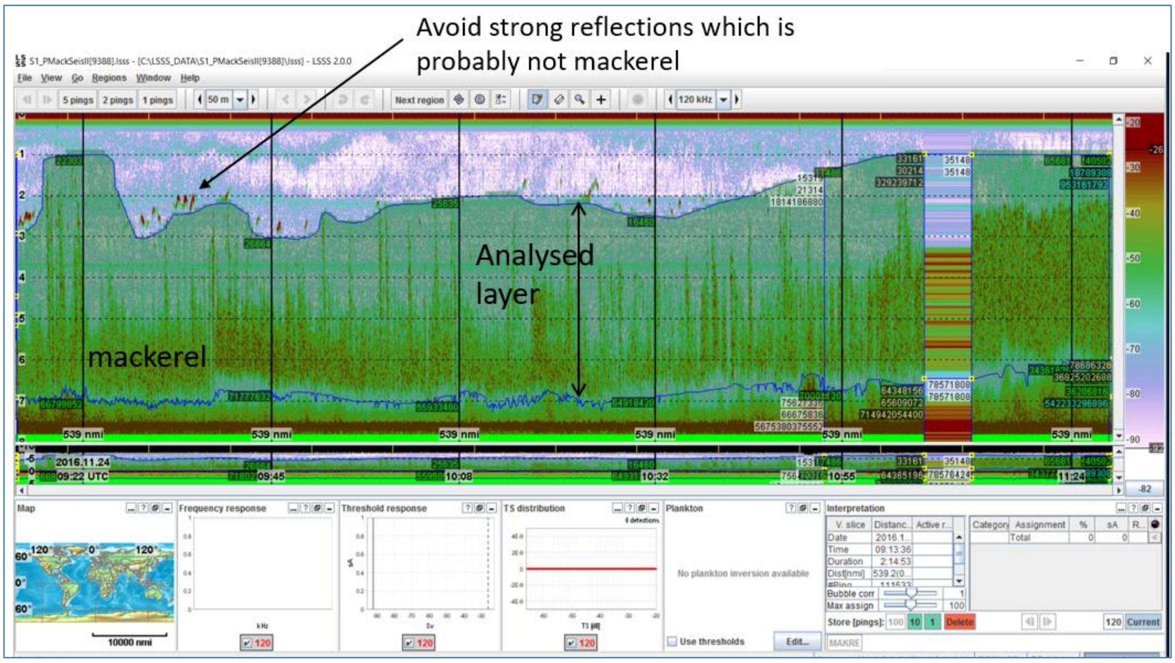

Figure 14. *Example of echosounder data in the software LSSS where the data used for the analysis is seen between two blue lines. The water surface can be seen at the bottom of the figure. The blue lines are drawn manually to avoid unwanted reflections.*

Three time-dependent variables were extracted from the echo sounder datasets:

1) Total sv. This is the total amount of scatterers in the water column covered by the echo sounder. The entire school is not covered by the beam, so when the fish swim around in a carousel pattern, different fishes will be covered by the echosounder at different times.
2) Mean depth: This is the depth weighted by the scatterers: For each time-index, the cumulative sum of (sv for each depth index multiplied with the corresponding depth) are divided by the sum of sv in the column: Cumsum(sv*range)/sum(sv), estimated for each time index.
3) Spread in depth: the depth range where 90% of the fishes are distributed. The cumulative sum over sv for each depth index are normalized to 1 by dividing by the total sv of the water column. Then the depth indexes where the normalized cumulative sum is larger or similar to 0.05, and smaller or similar to 0.95 are found. 0.05<cumsum(sv)/max(cumsum(sv)<0.95

Each of these three sizes are smoothed with a moving mean of 11 points. The echo sounder stopped several times during the measurements. It was then started again as soon as possible. This have caused many gaps in the dataset. For the smoothed data, 5 point to each side of the gaps were set to NAN (not a number) after the smoothing. Figure 15 shows an example of the three variables from the echosounder measurement for Block 6. Variations in mean depth or spread indicate a vertical movement of the school, or a change in school structure, typical behavioural reactions of fish schools to potential stressors such as sound exposure. The total sv is used mostly as a test together with the other curves to ensure that the mean depth or spread in depth is based on a reasonable number of fish (that there is fish within the beam).

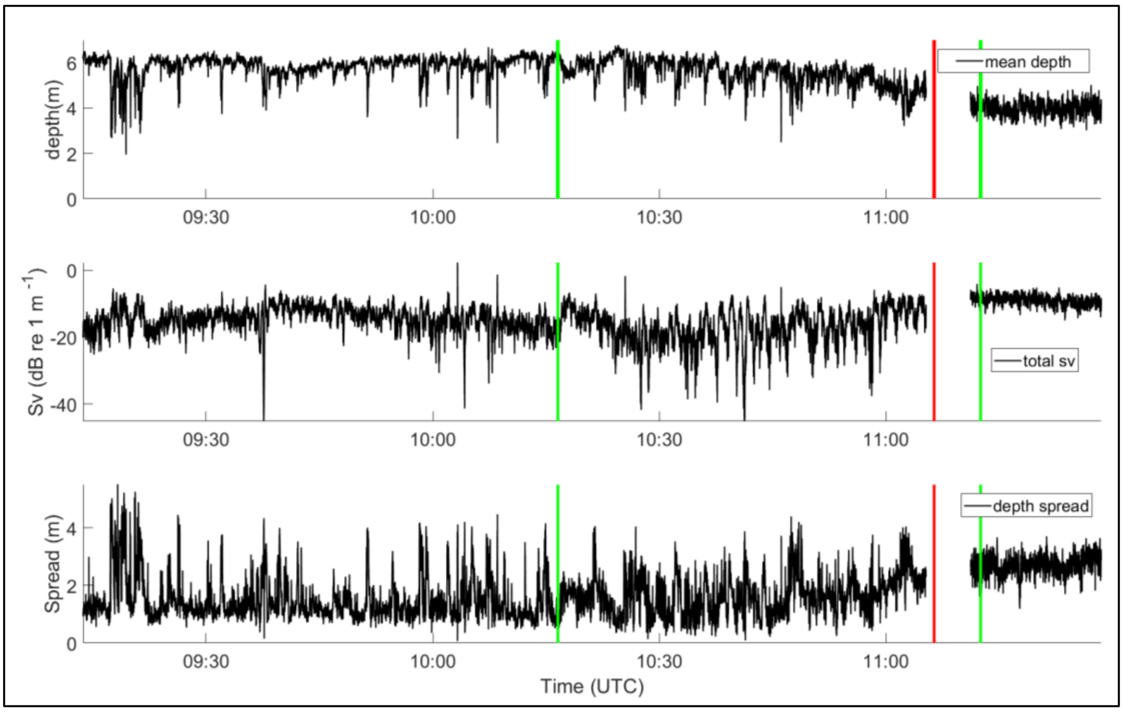

Figure 15. *Example of dataset extracted from echosounder data for block 6. Green lines mark start and stop time for seismic shooting. Red line marks the closest point of approach. Time is given as UTC.*

2.4.3 Data exploration, analysis and statistics

One of the main aims of this study was to identify levels of sound pressure/particle acceleration of which a behavioural response was induced. The first data exploration therefore was to plot the time sequences for all measurement instruments for individual blocks and runs, and as a first step carefully studying these to try identifying obvious changes by eye. However, no sudden or abrupt change in behaviour could be identified. This was also the impression from videomonitoring in real time during the experiments.

Statistical analyses of long time series like those collected here are challenging, in particular due to the autocorrelation of the measured data. To avoid this issue, we therefore blocked data in time bins and compared these.

For swimming speed, data for the two dose escalation blocks analysed (6 and 7) was divided in time bins of 5 min each; 5 min before exposure started (Pre), 5 first min of the exposure (start) the 5min surrounding the closest point of approach (CPA) and 5 min after ended run (Post). For the swim speed measured during exposure (Start and CPA), only measurements done during the pulses were used.

The same division was done for videoscoring, for the dose escalation blocks analysed here (blocks 2, 6, 9 and 10).

For echosunder data, 3 min bin were selected as 3 min prior to start (Pre), 3 first min into exposure (Start), at CPA (CPA), the last 3 min of exposure (Stop) and 3 min period after ended exposure (Post).

Differences between these selected periods was tested with an ANOVA test was conducted to compare the different phases. This was done in R (www.r-project.org) by using the *aov* and *TukeyHSD* commands.

3 Data collected

The cruise was over 7 days, but due to weather limitations, only 5 had workable conditions (Table 2). Over these 5 days, 3 different batches of mackerel were exposed, and a total of 11 blocks and 17 runs was conducted (Table 3).

Mackerel had ha mean length and weight wit standard deviation of 41.7±2.8 cm and 863±163 g.

Table 2: *Overview of operation.*

Date	Description	Exposures conducted
7-11. nov.17	Testing of equipment, Austevoll research station	0
18.nov.17	First net pen towed to experimental location.	0
21.nov.17	Start of survey	1 control
22.nov.17	Regular operation	2 seismic dose escalation
23.nov.17	Regular operation	2 seismic dose escalation, 3 seismic short, 1 control
24.nov.17	Regular operation	2 seismic dose escalation, 2 seismic still
25.nov.17	Regular operation	2 seismic dose escalation, 2 seismic still close
26.nov.17	No operation due to weather limits	0
27.nov.17	No operation due to weather limits, packing of equipment	0
28.nov.17	Last pen towed back to Austevoll research station.	0

Table 3: *Overview of all transects with timing and closest point of approach (CPA) from the net pen.*

Date	Batch	Block	Run	Transmission type	Start	Stop	CPA time	CPA distance	# of pulses
21.11.2016	1	1	1	Control no seismic	13:38:00	14:43:05	14:40:36	300 m	0
22.11.2016	1	2	1	Seismic dose escalation	11:28:44	12:24:20	12:17:44	300 m	333
22.11.2016	1	3	1	Seismic dose escalation	13:19:00	14:16:00	14:09:39	300 m	342
23.11.2016	1	4	1	Seismic dose escalation	10:31:00	11:23:04	11:17:55	280 m	312
23.11.2016	1	5	1	Seismic short	12:59:26	13:06:06	13:03:20	220 m	40
23.11.2016	1	5	2	Seismic short	13:14:17	13:21:00	13:18:40	180 m	40
23.11.2016	1	5	3	Seismic short	13:28:05	13:34:02	13:46:38	250 m	40
24.11.2016	2	6	1	Seismic dose escalation	10:16:33	11:12:31	11:06:22	328 m	335
24.11.2016	2	7	1	Seismic dose escalation	12:32:00	13:31:02	13:24:36	321 m	354

24.11.2016	2	8	1	Seismic short	13:49:31	13:55:52	-	341 m	40
24.11.2016	2	8	2	Seismic short	14:05:50	14:12:34	14:09:46	298 m	40
24.11.2016	2	8	3	No seismic	14:18:53	14:24:53	-	-	0
25.11.2016	3	9	1	Seismic dose escalation	10:00.35	10.56:59	10:50:44	316 m	338
25.11.2016	3	10	1	Seismic dose escalation	11:45:00	12:43:29	12:37:27	320 m	350
25.11.2016	3	11	1	Seismic still close	13:17:45	13:18:27	-	100 m	3
25.11.2016	3	11	2	Seismic still close	13:58:27	14:04:58	-	90 m	40
25.11.2016	3	11	3	Seismic still close	14:19:53	14:26:44	-	90 m	40

During all transects, as well as some time before and after, data were collected both on the behaviour of the fish and on the acoustic environment in the net pen.

3.1 Measurements of sound

CTD-measurements were made once a day from the 21. to the 25. of November (Figure 7). There was not much variation between the readings from day to day. The temperature, speed of sound and salinity all showed maximum values between 66-72 meters depth. The last two days the depth of the maximum for speed of sound and temperature was at only 35-40 meters, while the maximum salinity was still around 62-65 meters (Figure 16). For the purpose of the current study, with the air gun towed at 7 m depth and the net pen being 10 m deep, the surface layer is of most relevance. The parameters of the upper layer did not change much between days and locations.

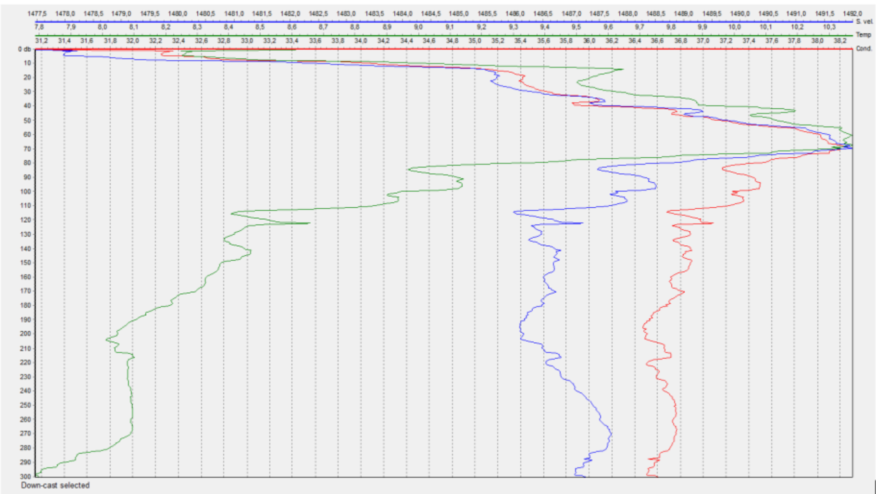

Figure 16. *Example of measurement from the CTD-instrument 23 Nov, showing sound velocity in blue, temperature in green and conductivity in red. All the measured parameters have maxima around 65 meters.*

3.1.1 Sound pressure

Sound pressure was measured outside the net pen with a hydrophone coupled on in 3 of the 5 working days (22, 23 and 24 Nov 2016), and has been analysed for blocks 6 (run 1) and Block 8 (run 1). Sound pressure inside the pen was measured at 3 and 5 m with the hydrophones at all working days (21-25 Nov 2016) and have also been analysed for Block 6 (run 1), Block 8 (run 1) and Block 11 (run 1 and 2), thus representing all the different types of exposure runs. As the sound field are not expected to change much between blocks/runs of the same type and are time consuming to analyse, these represented blocks were selected.

3.1.2 Particle acceleration
Particle motion sensor were measured in 3 of the 5 working days, specifically 22, 23 and 24 Nov 2016, and has been analysed for blocks 6 (run 1) and Block 8 (run 1).

3.2 Measurements of behaviour

3.2.1 Swimming speed
Measurements of swimming speeds were accomplished from video recordings of a single mounted video camera (i.e. not stereo camera). Our measurements were thus limited to the two spatial dimensions, x and y, but lacking the third, z, to generate three-dimensional coordinates. To minimise movements in the third dimension, we carefully selected fish which trajectory predominantly followed the two dimensional plane; i.e. fish were only tracked when passing approximately perpendicular to the camera view, moving parallel to the xy plane with its entire lateral side presented to the camera. Following these criteria, we were able to track and analyze swimming speeds in 111 still frame sequences, sampled, before, during and after the initial run with seismic airguns of block 6, 7 and 8 (Table 4). A list of all analyzed image sequences is presented in Appendix A.

Table 4. *Overview of image sequences were swimming speeds were analysed.*

	Block 6 - run1 (dose escalation)	**Block 7 - run1 (dose escalation)**	**Block 8 - run1 (seismic short)**
Pre exposure	6	4	4
Exposure - during sound pulse	17	17	9
Exposure - in between sound pulses	17	17	9
Post exposure	4	3	4
Total number of sequences	44	41	26

3.2.2 Vertical position and distribution in the pen
Echosounder data was measured 4 of the 5 working days. The 22. Nov 2016 the echo sounder was not working. Data has been analyzed for Block 6 (run 1), Block 7, (run 1), Block 8 (run 1,2 and 3) measured 23. Nov, and for Block 9 (run 1) and Block 10 (run 1) measured 24. Nov. Echosounder data was not measured for Block 2 and 3. The data from Block 4 was corrupt. For Block 5 there was hardly any fish visible on the echo sounder. Data from Block 1 and 11 have not been analyzed yet. EK 60 data was collected in all blocks, except for block 2 and block 3. This was because the EK60 wide band transceiver (WBT) failed and we had to get hold of a new one. We also had some trouble with the new one, as it stopped recording and needed to be restarted from time to time, leading to some missing data points. However, most of the time from the experiments we have sufficient echosounder data.

3.2.3 Videoscoring
Scoring was done for all the different types of exposure, representing all the four different batches, elaborated in table 5.

Table 5: *Overview of blocks and runs that has been scored.*

Date	Block	Run	Batch	Exposure type
22.11.2016	2	1	1	Dose escalation
24.11.2016	6	1	2	Dose escalation
25.11.2016	9	1	3	Dose escalation
25.11.2016	10	1	3	Dose escalation
23.11.2016	5	1	1	Seismic short
23.11.2016	5	2	1	Seismic short
23.11.2016	5	3	1	Seismic short
24.11.2016	8	1	2	Seismic short
24.11.2016	8	2	2	Seismic short
23.11.2016	5	4	1	Control, no seismic
25.11.2016	11	1	3	Seismic still close
25.11.2016	11	2	3	Seismic still close
25.11.2016	11	3	3	Seismic still close

3.2.4 Positive and negative controls

Two control runs were done with the source vessel transiting along the transect, towing the air gun, but without shooting. This was done for one dose escalation (Block 1, run 1) and one seismic short (Block 8, run 3). The main aim with these was to act as a negative control, to be able to separate any behavioural change to the seismic sound to that of the vessel itself. However, since we did not find any obvious change in behaviour during the runs with seismic, these have not been analysed in detail.

Block 11, seismic still close, were intended as a positive control, to see if we could trigger a response by increasing the received sound level, as well as getting the maximum level from the first pulse. This block consisted on 3 runs. The first was only 3 seismic pulses, as it had to be aborted due to the air gun being too close to Mosby. In run 2 and 3, the source was tan placed further from Mosby, at a distance of about 90 from the net pen. Both these runs had 40 seismic pulses. We did observere the fish to respond by swimming up and down the net pen, as can be seen on the echograms (Figure 17). In run 1, we see that the start this behaviour at start of exposure, but in run 2 they are continuing this from block 1, so no change in behaviour at start of exposure in run 2.

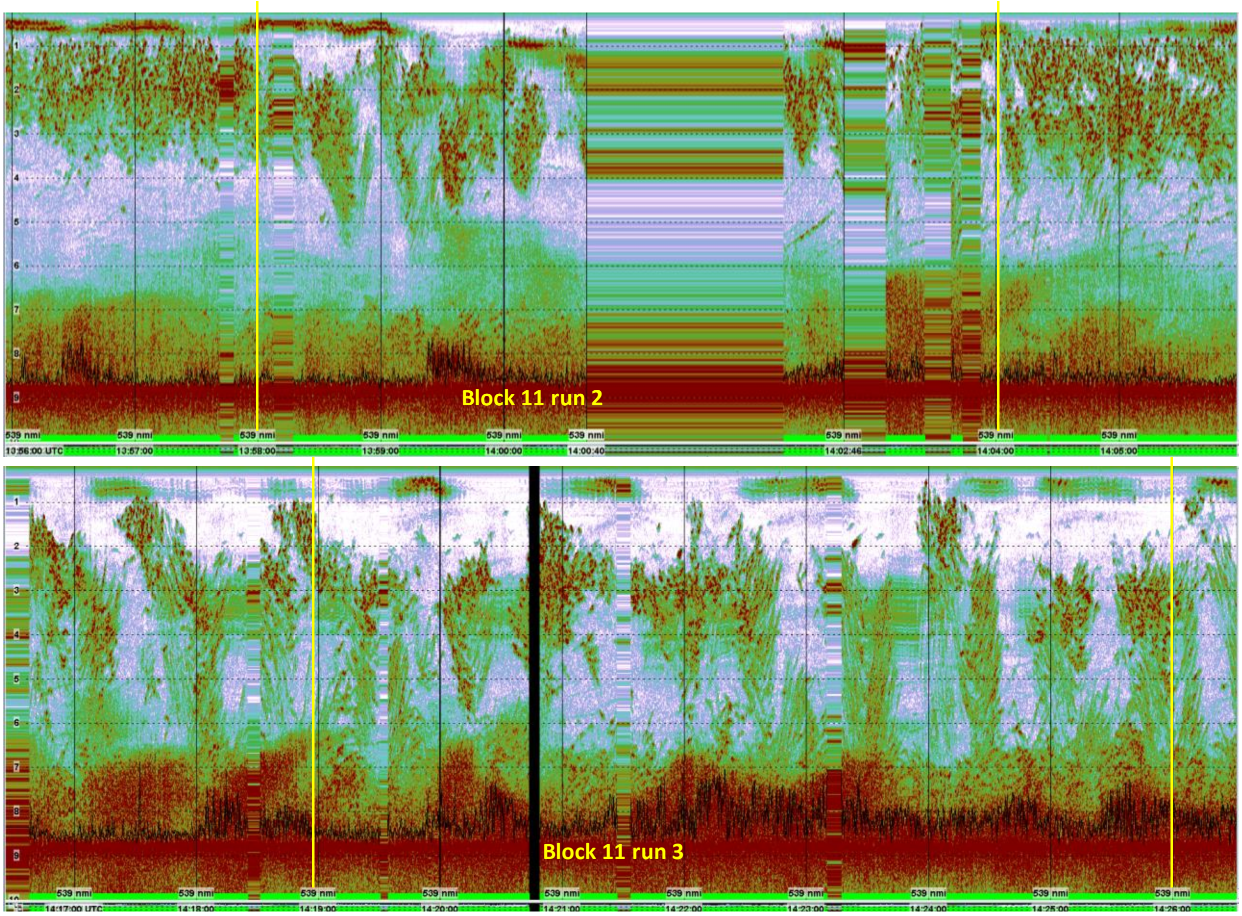

Figure 17. *Echogram for block 11, runs 1 and 2. Gaps indicate missing data.*

In addition, we have a few instances of non-planned positive controls. The fish did in several cases show intense reactions to incoming waves from ships passing. An example is for block 9, at 10:17 UTC, some large incoming waves from a passing large passenger ship was noted in the log. Echosounder and video show a clear response from the mackerel, with an abrupt change in vertical position (Figure 18) and a change in score from calm, carousel swimming with medium coordination to sprint, searching mode with high coordination (Figure 18) (see also Figure 44 for block 9, showing a short term change in scoring parameters around time 165-170 seconds after start).

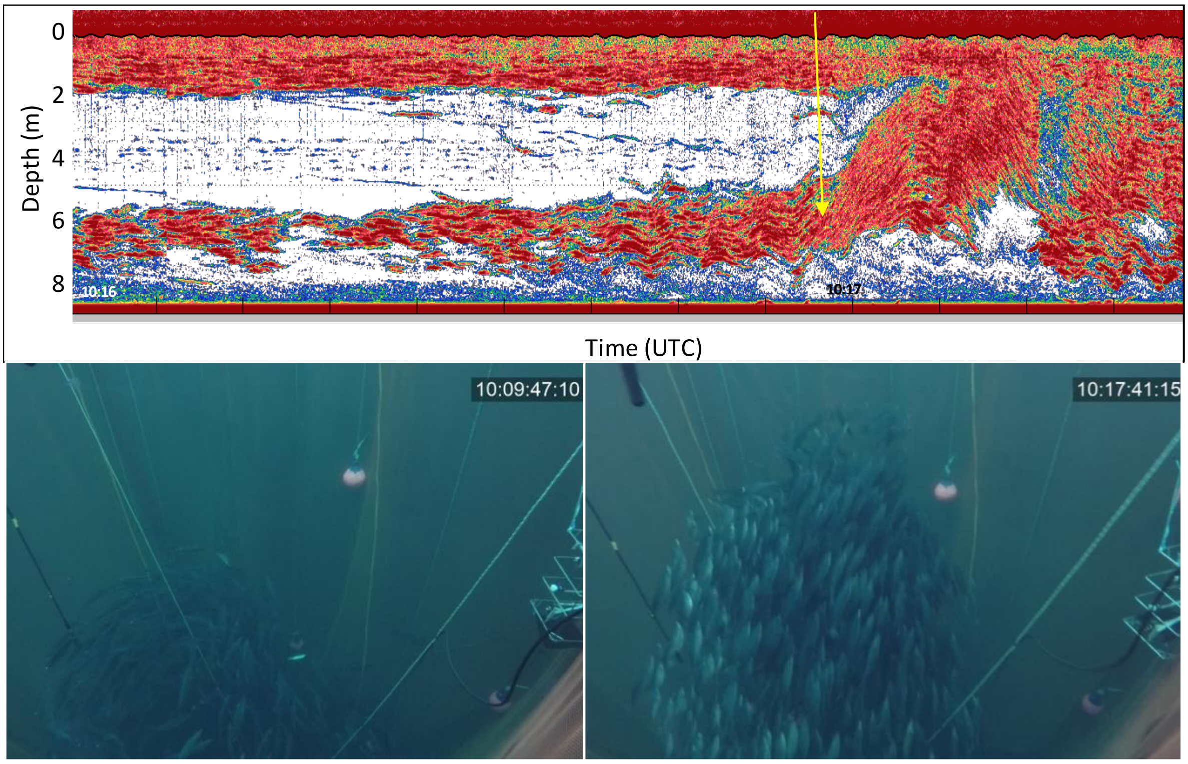

Figure 18. *Upper panel: Example of echogram showing a clear reaction from the mackerel due to incoming waves. Before the fish stay in a layer between 6-8 m depth, but as waves are hitting the pen (marked by yellow arrow), fish start move up and down in the pen. Lower panel: Example of pictures from before (right) and during reaction during block 9 at time 10:17:40.*

These examples show that the fish are fully capable of showing a behavioural response, as well as for our measurement systems to identify such responses.

3.3 Measurements at aquaculture farms

Fish behaviour was monitored three different fish farms, located at each side of the transect (Figure 19), thus all three being exposed to the seismic signal, especially during seismic dose escalation. The closest point of approach during seismic dose escalation was approximately 1 nautical mile (nmi) for two of the aquaculture facilities and 1.5 nmi for the third one.

One scientist was placed on one of these fish farms during all experiments. The scientist ensured filming of the video surveillance of all net pens of that particular farm, thus documenting a potential behavioural reaction of the farmed fish. The scientist was thus also continuously in dialogue with the employees at the fish farm, both for explaining the experiment as well as getting their opinion of whether the fish seen did show any form of reaction to the sound exposure.

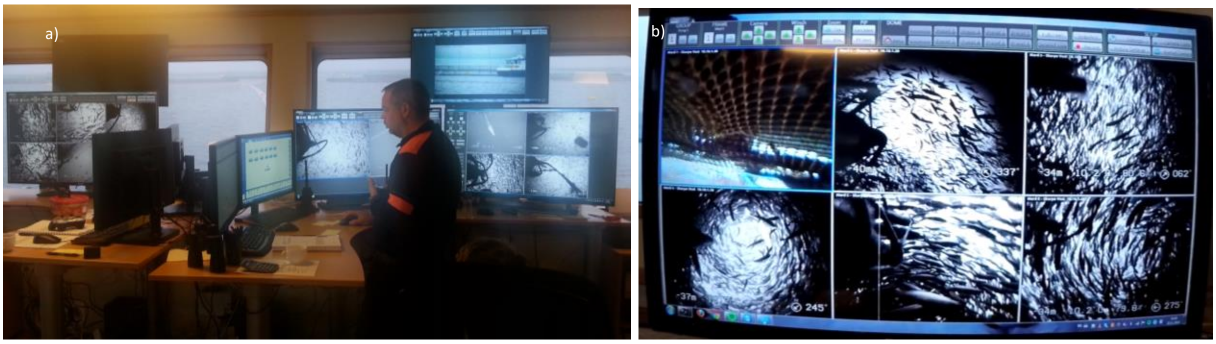

Figure 19. *surveillance at fish farm. a) video monitoring by employee at the fish farm and b) screenshot from video material recorded, showing video surveillance in 5 different pens.*

4 Results

4.1 Sound pressure and particle acceleration

As described above, sound pressure and particle acceleration have been analysed in detail for one representative block for each of the exposure types; Block 6 (dose escalation) and Block 8 (seismic close). In addition, Block 11 (seismic still close) have been analysed for the hydrophones inside the pen. All of the seismic pulses, even the first ones about 7 km away were detected by the hydrophone. Figure 20 show an example, from block 6, of how the measured data with one of the hydrophones and gives an impression of the seismic pulses relative to the background noise. Additional figures for all blocks can be found in Appendix A.

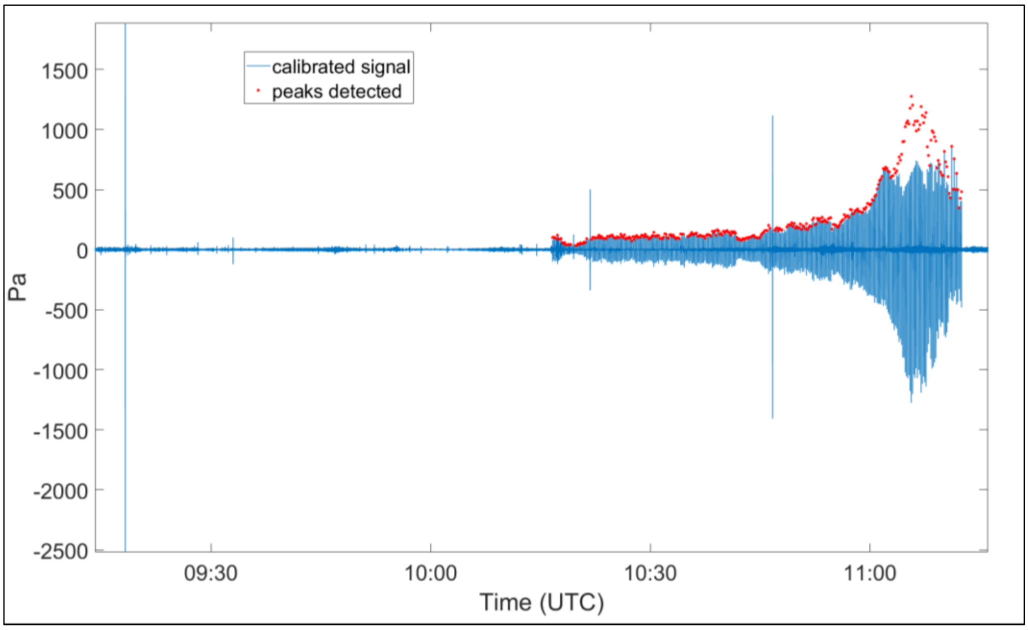

Figure 20. *Block 6, hydrophone 1 at 5 m depth. This is the entire time series which shows the detected sound before, during and after seismic exposure. The seismic pulses are the peaks marked with red dots. There are 10 seconds between each peak. Some noise peaks, not related to seismic, are also seen in the figure at random time intervals.*

4.1.1 *Block 6, measurements outside net pen (PM-sensor and Cetacean C55RS hydrophone):*

In Figure 21, ESD plots based on PM-sensor data are presented. Data is bandpass filtered in the frequency range of 5-1000 Hz to show the full bandwidth of the signal. The six curves in the figure show data from different distances and times from block 6. The solid lines are from seismic pulses from three different distances. Blue: Pulse 1, the distance is maximum (~7000 m). Red: Pulse 188, the distance is roughly half the distance from maximum based on 02P levels. Green: Pulse 300, CPA distance (~330 m). The dash dotted lines are the corresponding background levels measured 1-2 s before each seismic pulse.

In Figure 22, ESD plots based on hydrophone data are presented for the corresponding airguns pulses as in Figure 21. Comparing Figure 21 and Figure 22, one can conclude that the main energy of the seismic gun lies below 400 Hz. The low frequency content (<25 Hz) in the PM-sensor data is at some level originating from the coupling of the sensor to vessel, i.e. vibrations from the vessel is transmitted through the rope to the sensor. The sensors noise floor can also contribute here below some frequency. As mentioned earlier the sensors noise floor at 10 Hz is 4 $\mu g/Hz^{1/2}$= 32 dB re 1 $\mu m/s^2$ or translated to ESD level ~44 dB re $(1\ \mu m/s^2)^2 \cdot s$. The noise floor rapidly falls of to 3 dB re $(1\ \mu m/s^2)^2 \cdot s$ at 100 Hz.

One can also notice that the PM-sensor seems to be more sensitive in the higher frequency domain (>400 Hz). The PM-sensor data shows a different frequency content with higher signal levels compared to the hydrophone data. This could be due to the reason that there are acoustical near field effects that are not resolved with a hydrophone (the sensors are only 5 m from the water surface). The reason could also be that we are overestimating the sensitivity in the higher frequency range.

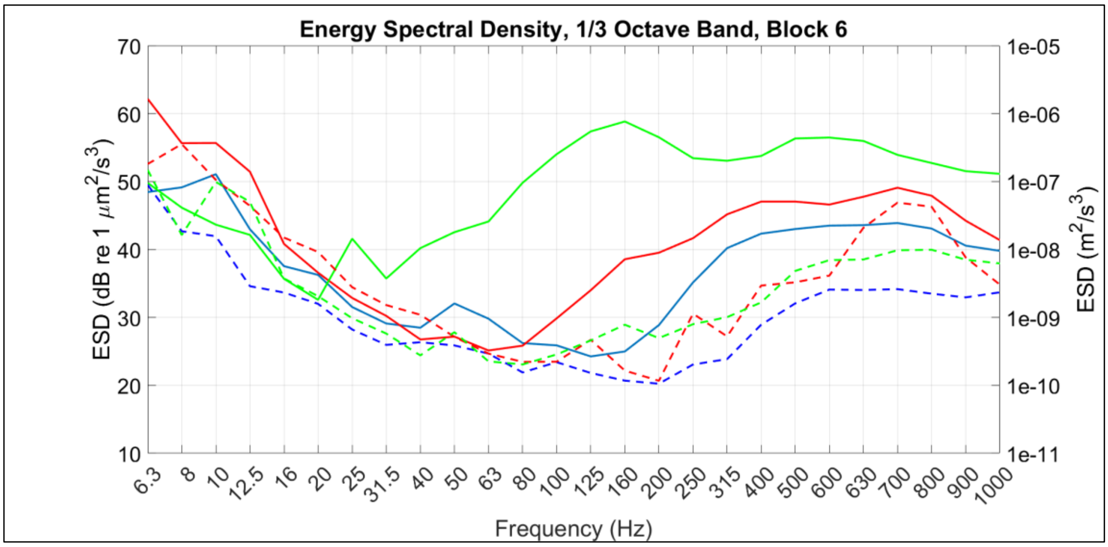

Figure 21. *ESD plots based on PM-sensor data. Blue: Pulse 1, the distance is maximum. Red: Pulse 188, the distance is roughly half the distance from maximum based on 02P levels. Green: Pulse 300, CPA distance. The dash dotted lines are the corresponding background levels measured 1-2 s before the seismic pulse.*

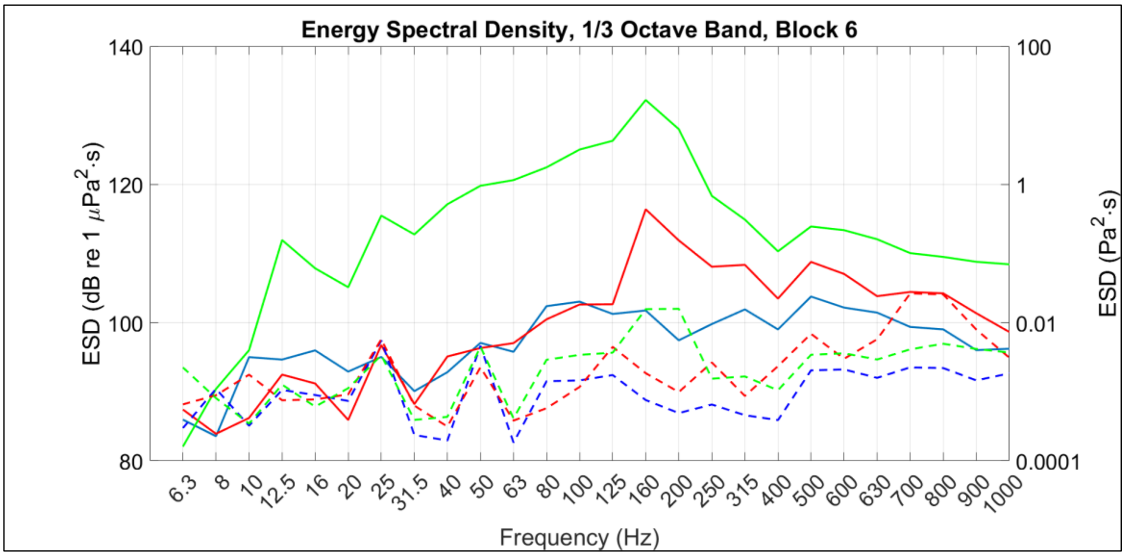

Figure 22. *ESD plots based on hydrophone data. Blue: Pulse 1, the distance is maximum. Red: Pulse 188, the distance is roughly half the distance from maximum based on 02P levels. Green: Pulse 300, CPA distance. The dash dotted lines are the corresponding background levels measured 1-2 s before the seismic pulse.*

In order to study the increasing level of noise as the vessel approached the net pen, 02P values for the particle motion are presented as a function of the pulse number (Figure 23a). This data is bandpass filtered in the range of 5-400 Hz, as to give the frequency range actually sensed by the mackerel. The gaps in the plot are due to the reason that at times there was so much heaving of the vessel that it distorted the data from the PM-sensor. CPA at pulse 300 can be easily seen on this plot.

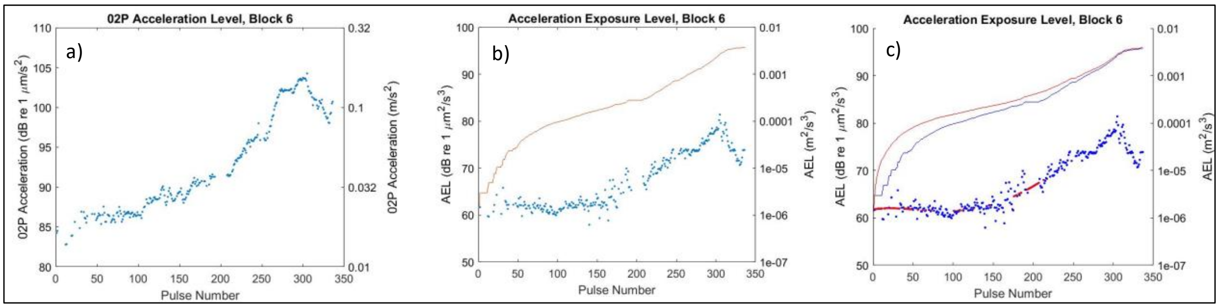

Figure 23. *a) Zero-to-peak (02P) values are presented as a function of the pulse number. b)AEL levels are plotted as a function of the seismic pulse number (blue dots). The red curve that show the cumulative sum of the AEL's. c) AEL levels are plotted as a function of the seismic pulse number (blue dots). The red dots are symbolizing the missing pulses estimated from a polynomial fit to the blue dots. The (solid) red curve shows the cumulative sum of the AEL's when the energy is added from the fitted data. The (solid) blue curve shows the cumulative sum of the measured AEL's (blue dots).*

In Figure 23b, AEL levels are plotted in the same way as a function of the seismic pulse number (blue dots) together with a red curve that shows the cumulative sum of the AEL's. This data is passband filtered in the range of 5-400 Hz. In order to see how the removed data points (due to disturbance from heaving) could affect the cumulative sum of the energies an estimation of the loss was done. A 4th order polynomial was fitted to the data in the range from pulse 1 to 300. From this fit it was estimated how much (in average) energy the missing pulses would give (Figure 23c). The red dots are symbolizing the missing pulses estimated from the fit. The (solid) red curve shows the cumulative sum of the AEL's when the energy is added from the fitted data. The difference with the curve neglecting (blue curve) these fitted data is not that big when reaching the final pulse number 337.

The 02P values of the sound pressure as a function of pulse number is presented in Figure 24a, and SEL values are plotted as a function of the seismic pulse number (blue dots) together with a red curve that showing the cumulative sum of the SEL's in Figure 24b. These data are passband filtered in the range of 5-400 Hz.

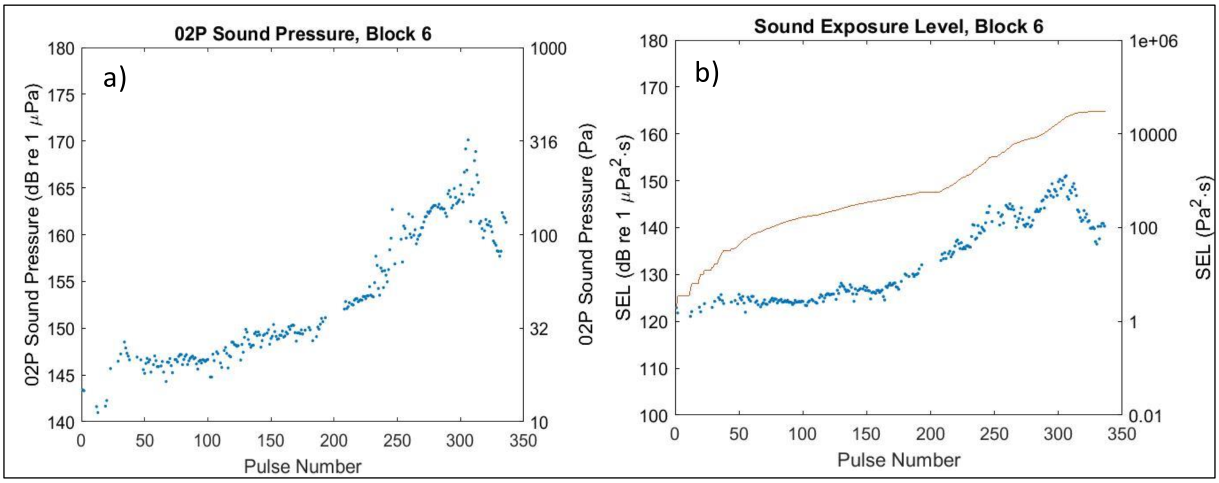

Figure 24. *a) 02P values of the sound pressure as a function of pulse number. b) . SEL plotted as a function of the seismic pulse number (blue dots) together with a red curve that showing the cumulative sum of the SEL's.*

SPL ranged from 143 dB re 1 µPa at a distance of 7000 m to 169 re 1 µPa at CPA of 330 m, while corresponding SEL values are 123 to 149 dB re 1 µPa^2 s (Table 5). Note that the SEL values in Table 6 is estimated after the 5-400 Hz bandpass filter was applied in order to find the relevant SEL for the fish hearing.

Table 6: *Block 6, sound measured outside net pen: Sound acceleration and pressure given for the distances of the first shot (7000 m) and CPA (330 m).*

Distance	Accelaration zero-peak (dB re µ Pa ms^{-2})	Accelaration zero-peak (ms^{-2})	Accelaration SEL (dB re µ Pa ms^{2}s^{-3})	Sound pressure level, zero-peak (dB re 1µPa)	Sound exposure level (dB re 1µPa^{2}s)
7000 m	84	0.02	62	143	123
330 m	103	0.15	80	169	149

4.1.2 Block 6, measurements inside the net pen (Two Brüel & Kjær hydrophones):

The sound measurement in Block 6 started about one hour before the start of seismic exposure. Measurements were made at two depths inside the net pen. The deepest hydrophone gives the highest peak values. Therefore, for the rest of the results only plots from hydrophone 1 at 5 meters are presented.

Since we know that mackerel hear sounds in the frequency range about 5-400 Hz, the frequency content of the signals was of interest. It is already mentioned that the pulse shape and the frequency content of the signals changes with the distance from the source (Figure 11). One second long sequences of signal around the seismic pulses were analysed and compared for pulses at different distances (outlined further in Appendix A). The frequency distribution around CPA differ from the start and the midway of the run; the first part of the run the signal is more broadband, with most between 300 and 1600 Hz, while at CPA most energy is between 10-200 Hz. This is within the centre of mackerel hearing.

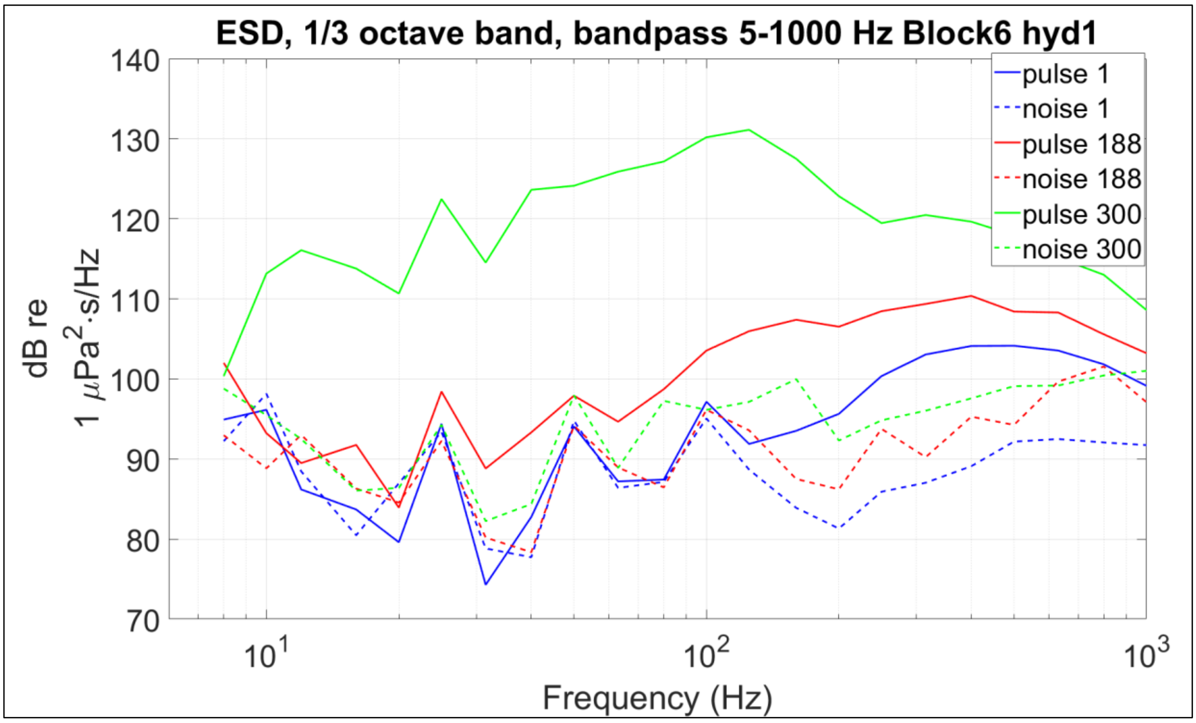

Figure 25. *ESD measured inside the net pen. Comparable to the result from the Cetacean hydrophone outside the pen (Figure 22).*

To compare the results measured outside the net pen (Cetacean hydrophone) to results measured inside the net pen with the (Brüel and Kjær hydrophone), the bandpass filter 5-1000 Hz was applied to the ESD plot (Figure 25). ESD is estimated for both one second around the seismic pulse and also for one second taken 2 seconds before the seismic pulse. The ESD level has a maximum around 134 dB inside the net pen while outside the net pen it was measured to 132 dB for pulse 300. ESD describe the distribution of energy in a pulse over the frequency range, while Sound Exposure Level (SEL) sums up the energy of the pulse, summed over time or frequency. The SEL is compared to the sound pressure peak amplitudes in Figure 26. Blue curves show the unfiltered signal, while red curves show the bandpass filtered result. For the sound pressure, there is a nearly constant difference between unfiltered and filtered results, but for the SEL (energy) the difference between the filtered and the unfiltered results get less as the difference between the source and receiver gets smaller. Around CPA the energy of the bandpass filtered results is similar to the unfiltered results. This can be explained by looking at the pulses in Figure 11. Much of the pulse 7 km away did not pass though the 4-500 kHz bandpass filter, resulting in energy of the filtered pulse and the unfiltered pulse to be quite different. For the pulse at CPA at 330 m distance much of the first sharp peak did not pass the filter, but almost all of the “rumbling” after the first peak passed through. The sharp first peak contributes little to the energy due to its short duration, therefore the energy before and after the bandpass filter is quite similar. Since fish hearing is in the range 5-400 Hz, this indicates that the fish can register most of the energy in the signal when it is at close distances (~1 km in this case), while for larger distances (>1 km) the fish does not hear all of the signal. It should be pointed out that the frequency content of the air gun will depend on the chamber size, and may differ between different airguns.

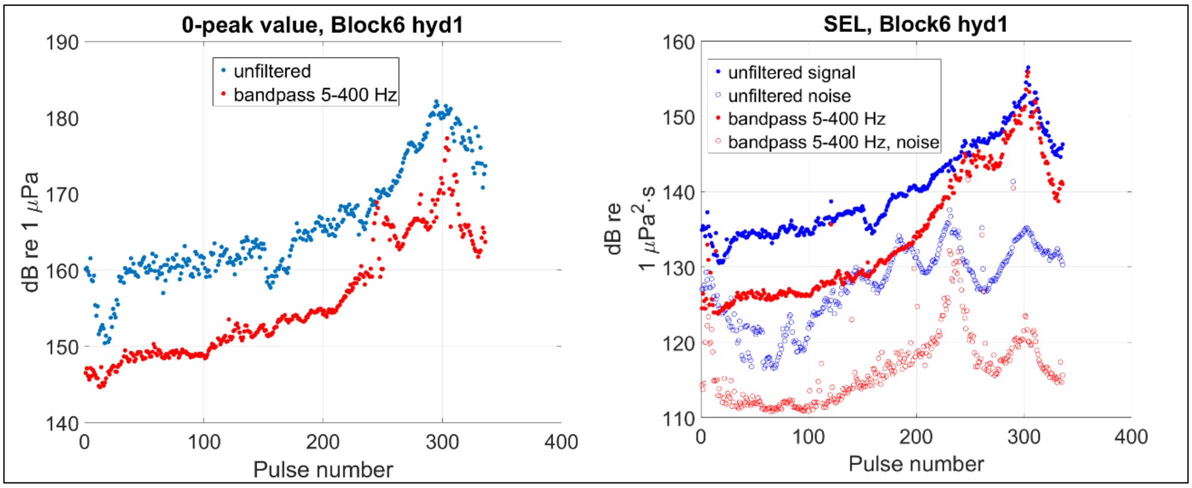

Figure 26. *Peak sound pressure given as the maximum peak value (positive or negative) relative to 1 uPa. Both unfiltered and filtered signals are used (left). Sound exposure level for each 1 second long sequence around a pulse. SEL for the noise is also estimated (right).*

A summary of some of the measured values for blocks 6, 8 and 11 are made in Table 7. The sound pressure in terms of Pa and as dB, and the sound exposure level are reported at the start of the seismic shooting, and at the closest distance. Both unfiltered and bandpass filtered results are listed.

Table 7: *Sound measurements inside the net pen measured with Bruel & Kjær hydrophones (SPL= sound pressure level, 02P=zero to peak, SEL=Sound exposure level.)*

			Fish hearing filter, bandpass 5-400 Hz			Unfiltered		
Block	Hydrophone	Distance	SPL 02P (Pa)	SPL 02P (dB re 1 µPa)	SEL (dB re 1 µPa^2s)	SPL 02P (Pa)	SPL 02P (dB re 1 µPa)	SEL (dB re 1 µPa^2s)
6	hyd 1	7000 m	21.2	146.5	124.5	101.5	160.1	134.9
6	hyd 1	330 m	369.6	171.4	150.9	1070.0	180.6	152.6
6	hyd 2	7000 m	12.3	141.8	120.5	75.9	157.6	132.4
6	hyd 2	330 m	350.8	170.9	151.0	758.6	177.6	152.0
8	hyd 1	~900 m	259.1	168.3	147.9	680.3	176.7	149.5
8	hyd 1	~300 m	819.7	178.3	159.2	1003.0	178.8	159.5
8	hyd 2	~900 m	150.5	163.6	146.0	437.2	172.8	147.3
8	hyd 2	~300 m	1004.0	180.0	157.8	1040.0	180.3	158.1
11	hyd 1	90 m	1619.0	184.2	160.0	3531.0	191.0	161.3
11	hyd 1	90 m	1503.0	183.5	160.1	3190.0	190.1	161.3

4.1.3 Block 8, measurements outside net pen (PM-sensor and Cetacean C55RS hydrophone)

In Figure 27, ESD plots based on PM-sensor data are presented. Data is bandpassed filtered in the frequency range of 5-1000 Hz. The graph contains six curves. The full lines are from seismic pulses from three different distances. Blue: Pulse 1, the distance is maximum. Red: Pulse 12, the distance is roughly half the distance from maximum based on 02P levels. Green: Pulse 25, CPA distance. The dash dotted lines are the corresponding background levels measured 1-2 s before the seismic pulse.
In Figure 28, ESD plots based on hydrophone data are presented. Data is bandpass filtered in the frequency range of 5-1000 Hz. The graph contains six curves. The full lines are from seismic pulses

from three different distances. Blue: Pulse 1, the distance is maximum. Red: Pulse 12, the distance is roughly half the distance from maximum based on 02P levels. Green: Pulse 25, CPA distance. The dash dotted lines are the corresponding background levels measured 1-2 s before the seismic pulse.

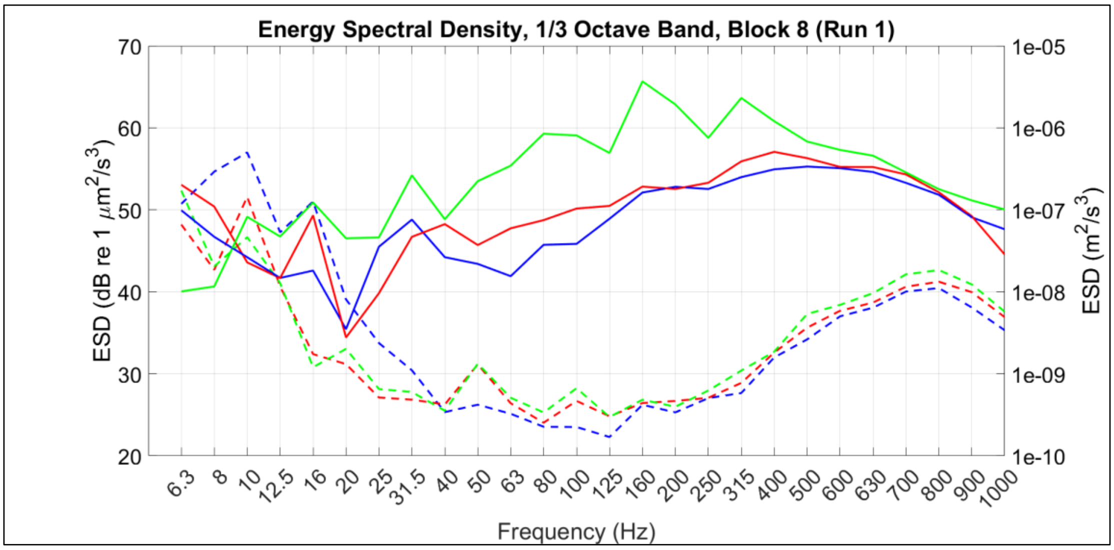

Figure 27. *ESD plots based on PM-sensor data. Blue: Pulse 1, the distance is maximum. Red: Pulse 12, the distance is roughly half the distance from maximum based on 02P levels. Green: Pulse 25, CPA distance. The dash dotted lines are the corresponding background levels measured 1-2 s before the seismic pulse.*

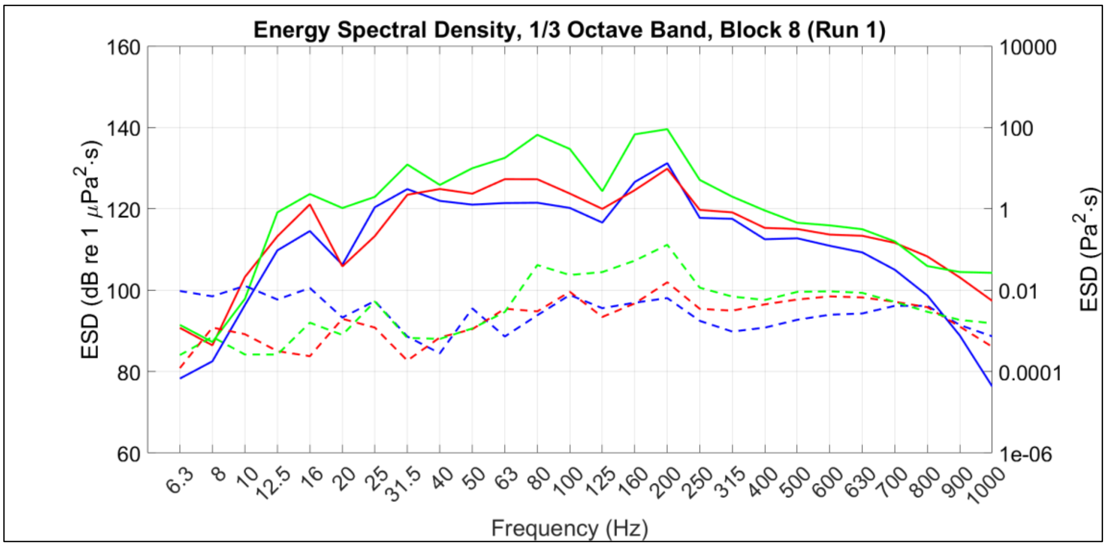

Figure 28. *ESD plots based on hydrophone data. Blue: Pulse 1, the distance is maximum. Red: Pulse 12, the distance is roughly half the distance from maximum based on 02P levels. Green: Pulse 25, CPA distance. The dash dotted lines are the corresponding background levels measured 1-2 s before the seismic pulse*.

In Figure 29, left panel, 02P values for the particle motion are presented as a function of the pulse number. This data is bandpass filtered in the range of 5-400 Hz. All pulses were measured successfully

in this Block and no points had to be removed. CPA seems to happen at around pulse number 25. There is a lot of variation in the energy of some pulses and the reason to this is unclear now. There is no heaving detected in these measurements. These variations could be attributed to the fact that sometimes the reverberation has a higher amplitude the main pulse, hence the automatic “peak detector” pick this as the peak level.
In Figure 30, right panel, AEL are plotted as a function of the seismic pulse number (blue dots) together with a red curve that show the cumulative sum of the AEL’s. This data is bandpass filtered in the range of 5-400 Hz.

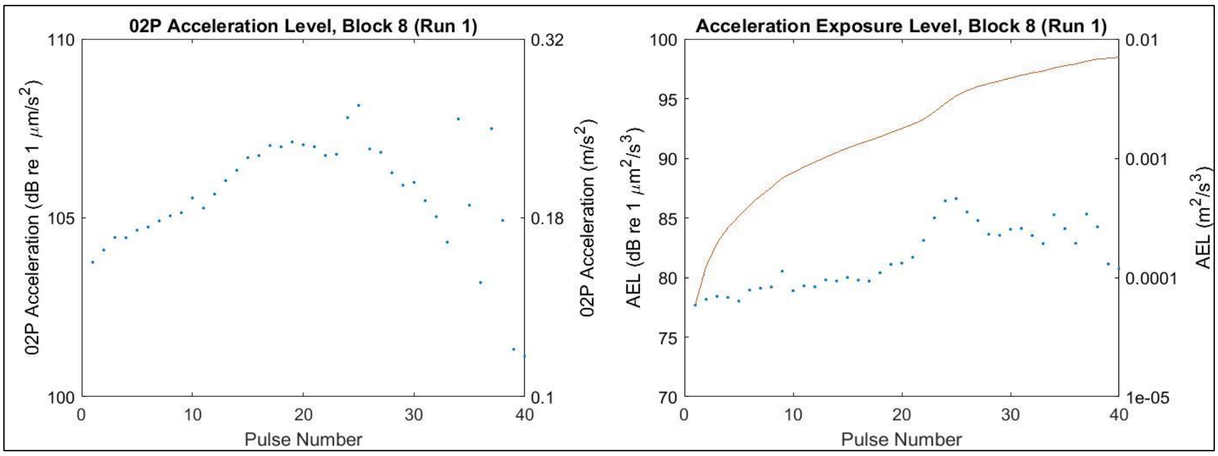

Figure 29. *02P values are presented as a function of the pulse number (left). AEL levels are plotted as a function of the pulse number (blue dots). The red curve that show the cumulative sum of the AEL’s (right).*

In Figure 30 left panel, the 02P values of the sound pressure as a function of pulse number is presented. This data is passband filtered in the range of 5-400 Hz. Here is a lot a variation in the energy after pulse number 23. In Figure 30, right panel, the SEL is plotted as a function of the seismic pulse number (blue dots) together with a red curve that show the cumulative sum of the SEL’s. This data is bandpass filtered in the range of 5-400 Hz.

SPL ranged from 178 Pa /165 dB re 1µPa at a distance of about 900 m, to 300-600 Pa/~170-175 dB re 1µPa at CPA of 300 m, while corresponding SEL values ranged from 145 to 157 re 1 µPa^s s m.

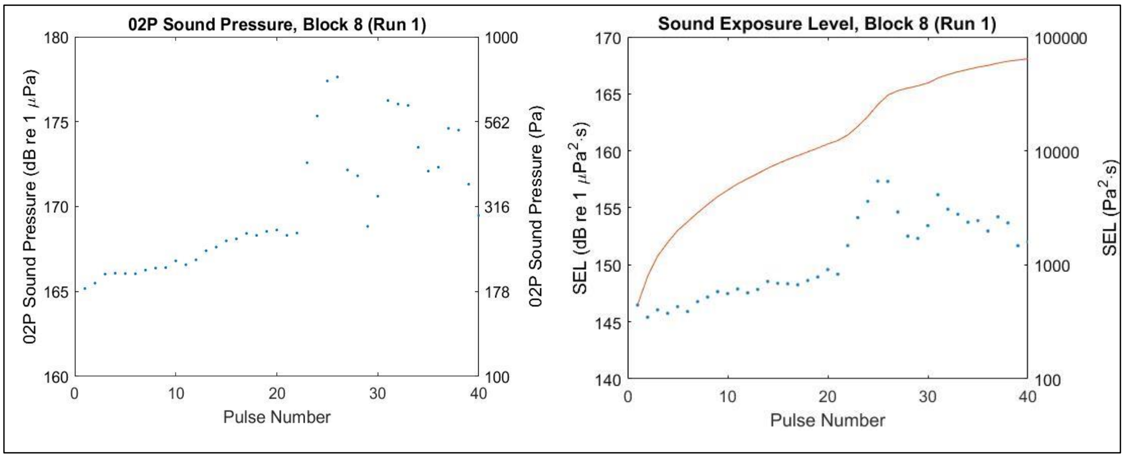

Figure 30. *02P values of the sound pressure as a function of pulse number (left). SEL plotted as a function of the seismic pulse number (blue dots) together with a red curve that showing the cumulative sum of the SEL’s (right).*

4.1.4 Block 8, measurements inside the net pen (Two Brüel & Kjær hydrophones):

Block 8 consisted of 3 runs, two short runs with seismic shooting, and one control with only the boat passing without seismic. The seismic shooting started at the same time as the hydrophone recording. Only results from run 1 is presented here. For block 8, run 1 the measurements are analysed in the same way as described above, and absolute peak pressure, ESD and SEL are compared to the measurements with the Cetacean hydrophone. The ESD measured with the Brüel and Kjær hydrophone (Figure 31) matches the similar measurement outside the netpen. Sound peak pressure and SEL (Figure 32) shows a similar trend as seen for the Cetacean hydrophone measurements with systematically increasing values in the first period of the run, while the last part of the run has more spread values. Both the level and the trends of the data points match well between the measurements inside and outside the net pen.

The main difference between Block 8 and Block 6 is the sound level at the start of the shooting. Block 6 started at a longer distance from the net pen, and therefor had lower sound pressure values which gave a gradual exposure. Block 8 started closer so the fish were exposed to rather high sound pressure values which gave a much more abrupt start of the sound exposure. From ss filtered results are listed.

Table it is seen that the peak pressure was 146 dB in the start of Block 6, while it was 20 dB higher in the start of Block 8. The SEL values were 124 dB in the start of Block 6, and 146 dB at the start of Block 8. The maximum sound pressure and SEL at CPA was also higher for Block 8 than for Block 6 probably due to the shorter closest distance for Block 8.

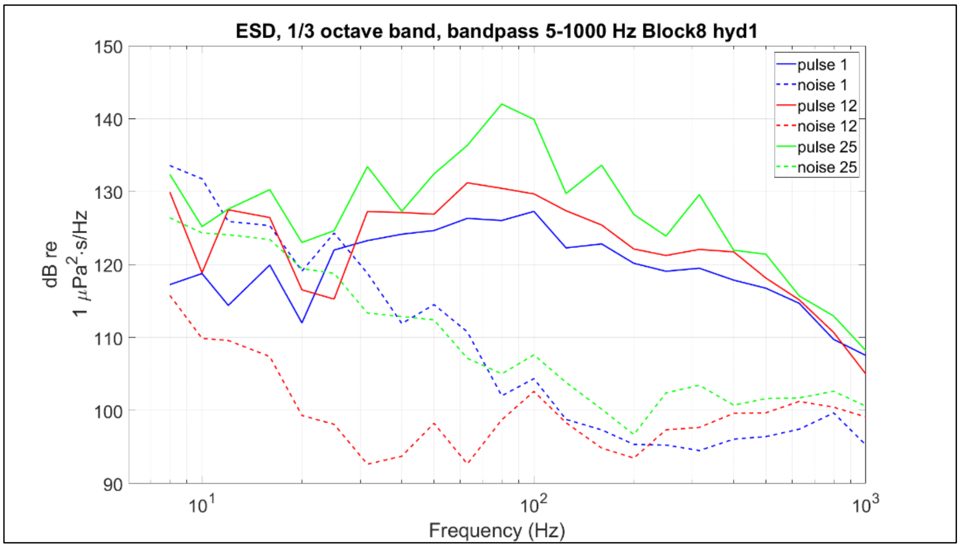

Figure 31. *ESD measured inside net pen for three selected shots, pulse 1: start of shooting at about 900 m distance, pulse 12: halfway, pulse 25: closest distance at about 300 m. This result is quite similar to the measurement made outside the netpen.*

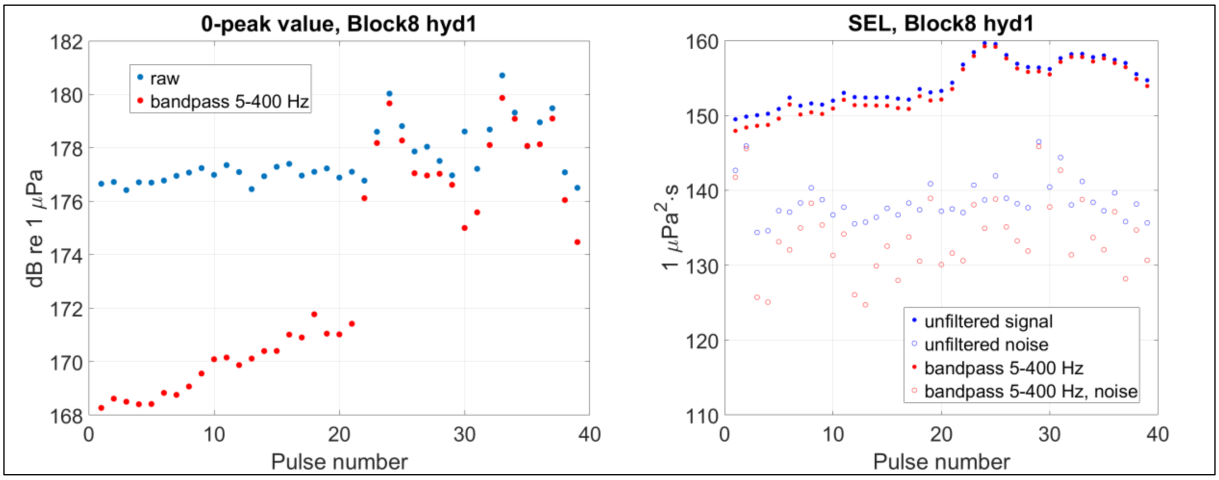

Figure 32. *Sound pressure level, and sound exposure level measured inside the net pen for Block 8, run 1. The red curves are bandpass filtered from 5-400 Hz. The blue curves are not filtered.*

4.1.5 Block 11, measurements inside the net pen (Two Brüel & Kjær hydrophones):

This block consists of 2 full runs with 40 shots each (run 2 and 3), in addition to a run of only three shots (run 1) that had to be aborted due to the air gun being placed too close to the source vessel. Here, the values for run 3 are shown. Here, all the 40 shots which was transmitted from the same distance. The positive peak is higher than for the negative peak for all the shots. This is the opposite of the CPA-pulses from Block 6 and 8. The amplitudes are around 3200 Pa or 190 dB for the unfiltered results. This is about 10 dB higher than at CPA for the other transect types.

The ESD for Block 11 is shown in Figure 33. The maximum energy is about 140 dB re 1 $\mu Pa^2 s/Hz$ around 80 Hz. This is not higher than for the other seismic exposures like Block 6 and Block 8. SEL which is estimated as the sum of the ESD for all the frequencies is a bit higher for Block 11. The SEL values for Block 11 is around 161 dB re 1 $\mu Pa^2 s$ (Figure 34). This is about 9 dB higher than for Block 6, but only 2-3 dB higher than for Block 8. The reason why there is a larger difference in peak amplitude than for ESD and SEL is that the pulse shape is a bit different between the three blocks. The CPA-pulses for Blocks 6, 8 and 11 are compared in Figure 35. This shows that the CPA pulse from Block 8 has more reverberation than the other Blocks. This can explain why Block 8 have a higher SEL than Block 6, and why it has SEL closer to the one of Block 11 even if the peak value is lower.

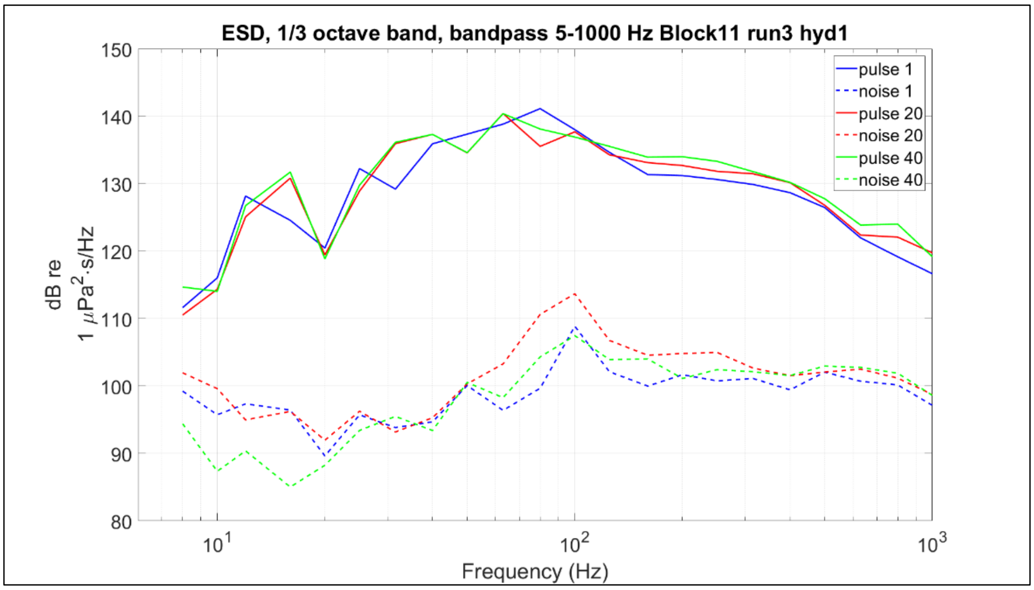

Figure 33. *ESD measured inside net pen for three selected shots, pulse 1, 20 and 40, all fired from the same distance; 90 m.*

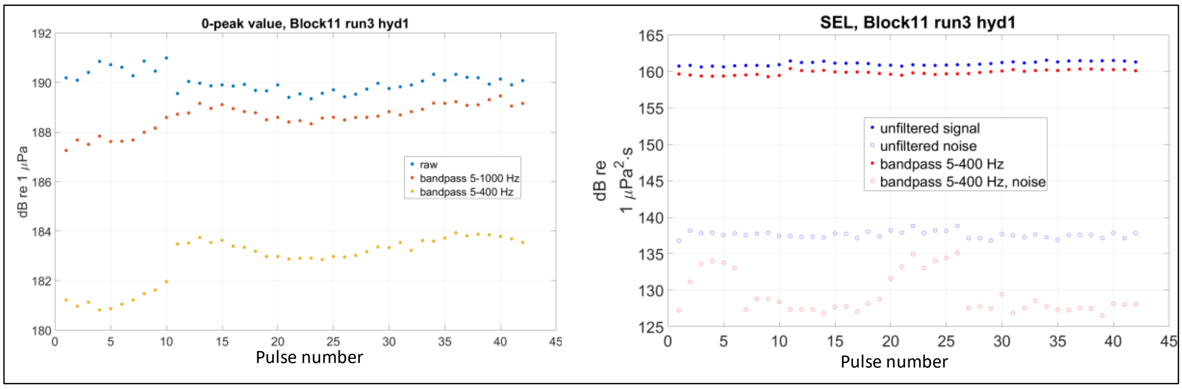

Figure 34. *Sound pressure level, and sound exposure level measured inside the net pen for Block 11, run 3. The red curves are bandpass filtered from 5-400 Hz. The blue curves are not filtered.*

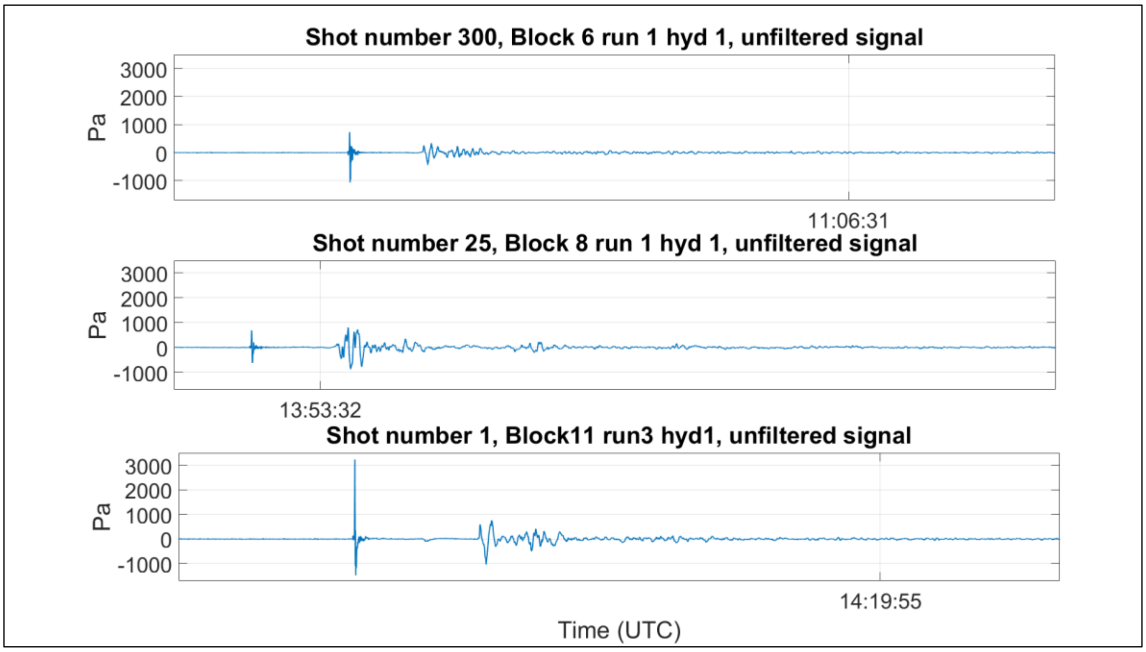

Figure 35. *Comparison of shots at the closest distance for three blocks. Top: Block 6, middle: Block 8, and bottom: Block 11.*

4.2 Swimming speed

Mean swimming speeds ranged from approximately 0.2 to 0.5 m/s, and was obtained by manually tracking the movements of 5 individual fish, in each of a 111 image sequences extracted from the video recordings. Swimming speeds measured during Block 6 is presented with respective SPL's in Figure 36.

The image sequence for the manual tracking can be found in Appendix D.

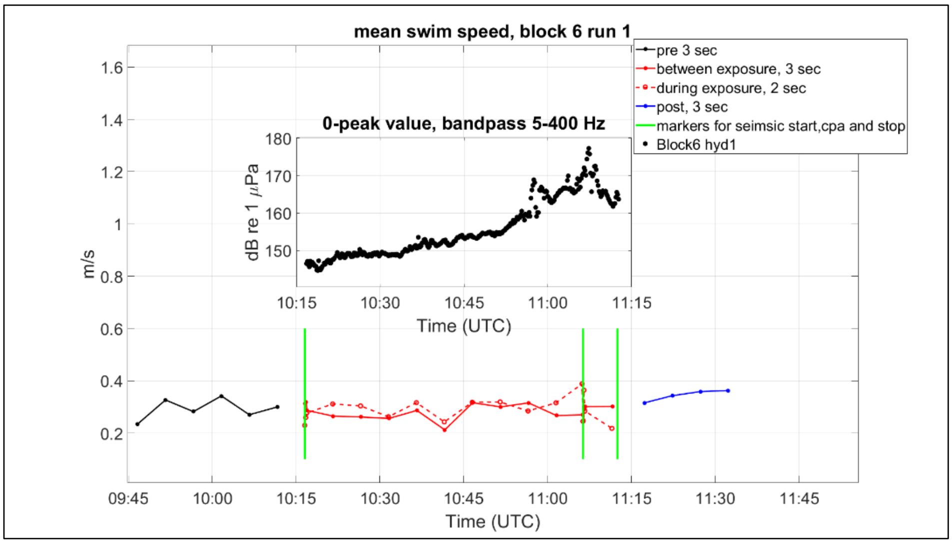

Figure 36. *Example of mean swimming speeds from Block 6 run1, as measured in 3 s duration image sequences from before (solid, black), during; i.e. in-between sound pulses (solid, red), and after (solid, blue) exposure to air gun sounds, and 2 s duration sequences during the sound pulses, starting at onset of the pulse (dashed, red). Sound pressure levels (SPL) presented for individual air gun pulses on a matching time scale, in the upper panel.*

Based on the three blocks analysed here, there does not seem to be any clear trend across the three experimental blocks (Block 6, 7 and 8) that could be attributed to responses to the initial airgun pulses. However, there is a potential, small tendency of swimming speeds increasing throughout the experiments, culminating in maximum speeds, around the time of CPA. Figure 37 compare the three periods Pre (before), During experiments and Post (after). As can be seen, there is a clear trend of the swimming speed to increase with block number; swimming speed increase with time after multiple exposures. There is also a trend of swimming speed to be higher in the Post phase for blocks 6 and 7 (dose escalation experiments). This trend can also be seen in the timeplot in figure 36.

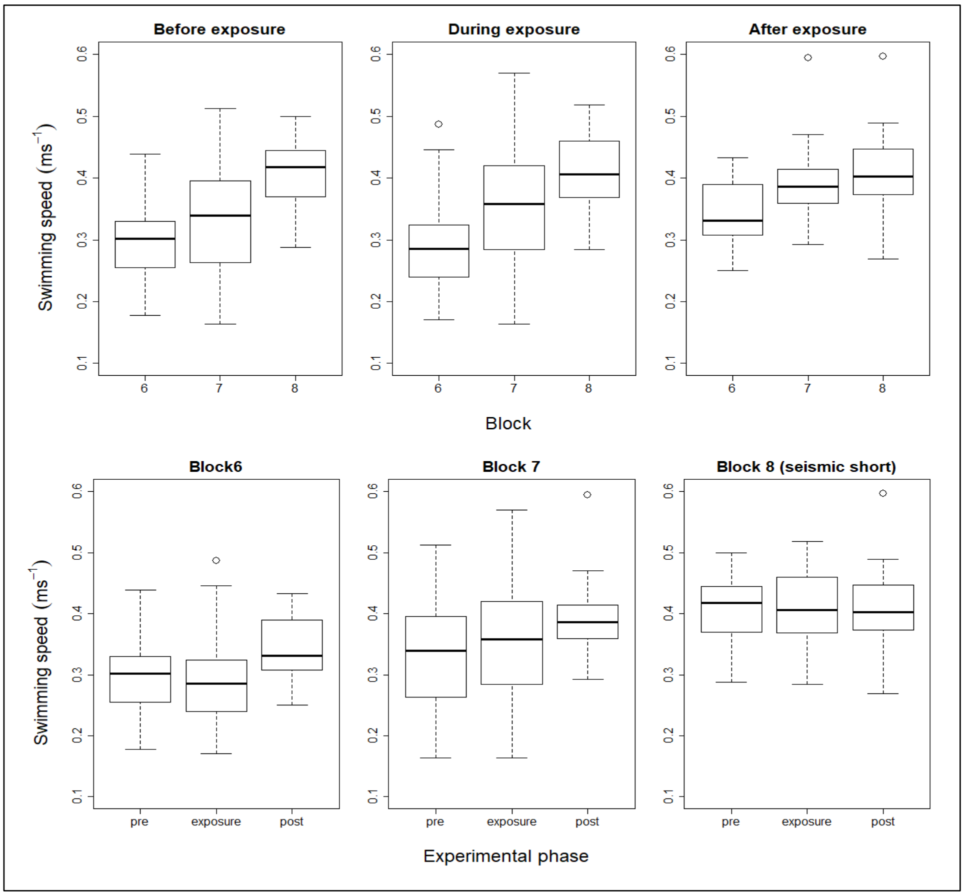

Figure 37. *Boxplot for swimming speed presented by experimental phase (upper level) and block (lower level). Swimming speed presented here are measured during seismic pulses.*

The results for the ANOVA comparison is shown in Table 8, and show the only significant difference in swimming speed to be the first 5 minutes of the exposure (Start) and the post exposure phase (Post). This is in agreement with the timeseries trends described above, that the swimming speed seem to increase throughout the period.

Table 8: *ANOVA comparisons for the phases Pre, Start, CPA and Post. Significant comparisons are marked in bold.*

Comparison	p-value
Post-CPA	0.675
Pre-CPA	0.779
Start-CPA	0.082
Pre-Post	0.277
Start-Post	**0.015**
Start-Pre	0.667

4.3 Effect on vertical position and spread of the fish

The vertical position and the spread of the fish turned out to be highly variable with time. The mean depth changes a lot even after being smoothed over 11 samples (about 1 second). Block 6, 7 and 8 was conducted succeeding on the same day and fish batch. Block 6 was thus the only of these where the seismic sound was novel for the fish. Just before the first pulse, a large passenger ship ("Danskebåten") passed and caused large waves hitting the pen, noted at 10:18:00. The upper subplot in Figure 38 shows the echogram for Block 6, with mean depth plotted on top. The middle plot shows the total sv, which is the amount of fish seen on the echosounder. The bottom subplot shows the spread in depth. Similar plots for blocks 7 and 8 can be found in Appendix B. A spread in depth of 2 means that 90% of the fish are distributed within a range of 2 meters. A slight change in vertical distribution can be seen just after the first seismic pulse, indicating downvard movement of the fish, potentially caused either by the seismic or the waves of the passenger ship. Vertical distribution again seem to change a few minutes before CPA, but unfortunately, the echosounder stopped recording until just before ended exposure. However, a trend in all three parameters from about 11:00; fish move deeper, and both total sv and spread increases with less variations. After the seismic shooting stopped, this behaviour continued, both for the 1 h 20 min break between blocks and into block 7, and no further changes are obvious during block 7 (Figure 39). A break of 20 min separated block 7 from block 8, with the latter being a seismic short run, in contrary to block 6 and 7 which both were dose escalations. Hence, in block 8, seismic exposure started at about 900 m as opposed to 7000 m for blocks 6 and 7, and with a correspondingly higher sound level from the first pulse. However, no obvious change in behavior seem to occur throughout this block, with the fish continuing the same mean depth, total sv and spread as since CPA of block 6. A time series plot of all the three blocks for this day are shown in Figure 39.

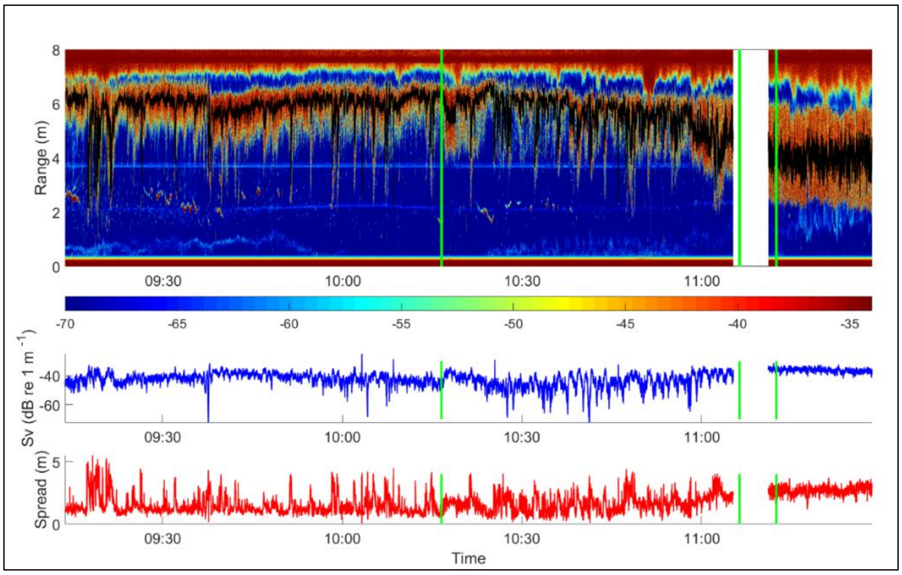

Figure 38. *Block 6: Mean depth is plotted as a black line on top of the echogram (upper level), the total sv (blue line) (middle panel), and the depth spread (lower panel). Start, max and stop of seismic shooting is marked with green vertical lines. White gap indicate missing data. "Range" in the echogram indicate the distance from echosounder, placed at approximately 8 m depth. The thick red colour at range 7-8 on top of the picture is thus the sea surface, while range=0 is about 8 m deep. Time is given as UTC.*

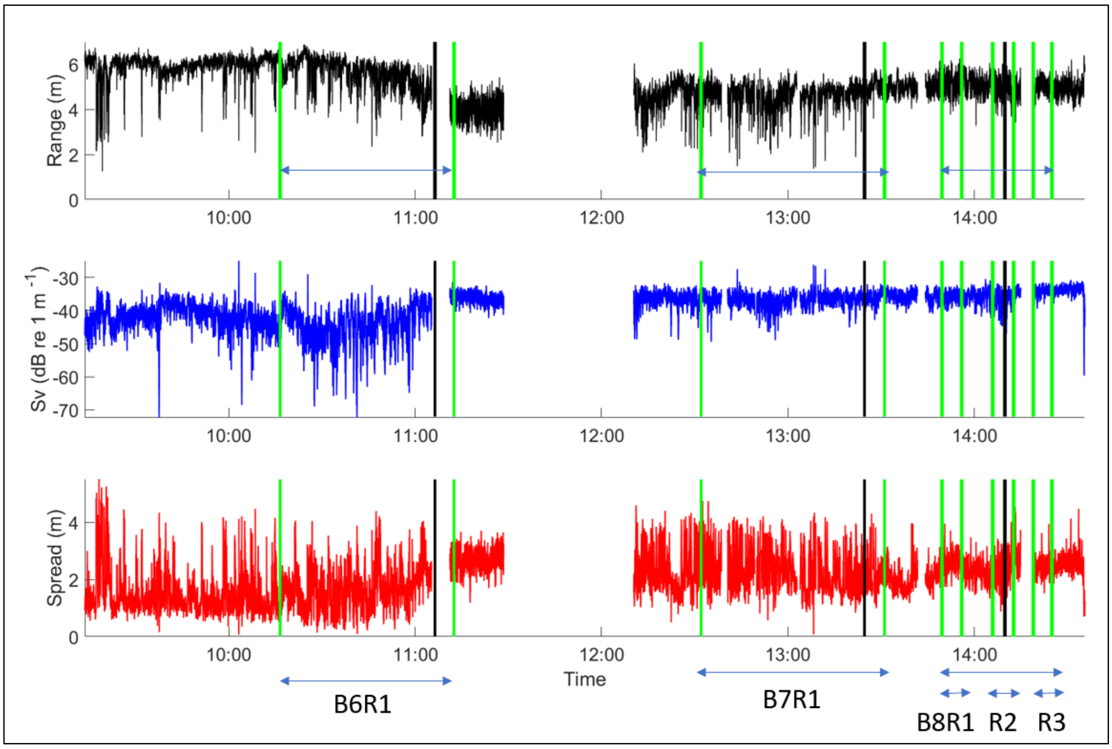

Figure 39. *Time series plot of Block 6, Block 7 and Block 8; mean depth (upper level), total sv (middle panel), spread in depth (lower panel).*

The results from Block 6,7 and 8 were all on the same batch of fish and measured the same day. These results can be compared to results from another batch of fish, measured the day after. Block 9 and 10 are the same type of exposures as Block 6 and 7, but here the fish were distributed in two layers. Echograms for these are shown in Appendix B. The layer closest to the bottom was analysed and can be compared to results from Block 6 and 7. Block 9 was thus the novel stimuli, as for block 6. The change in school structure seen at CPA of block 6 and lasting through blocks 7 and 8, was not seen in block 9. Block 9 and 10 was separated by a 50 min break. A time series plot for Block 9 and 10 is shown in 40. The results are quite variable with un-systematic large variations.

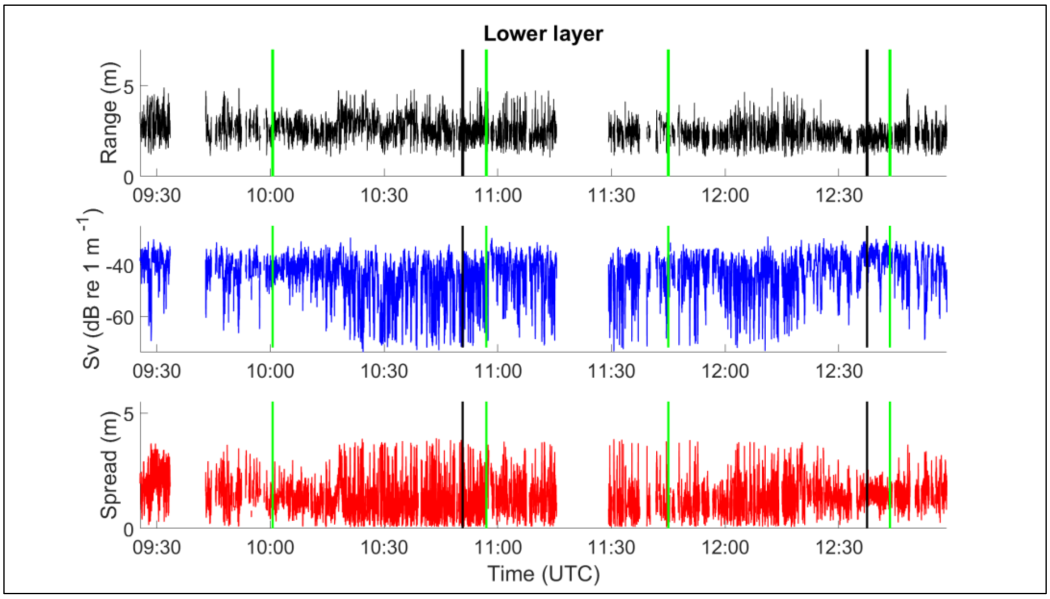

Figure 40. *Time series plot of Block 9 and Block 10; mean depth (upper level), total sv (middle panel), spread in depth (lower panel).*

The data were subdivided into successive time periods of interests (see section 2.4), comparing before (Pre), when the exposure starts (Start), at maximum exposure (CPA),at the end of the exposure (Stop) and after exposure (Post). Figure 41 show how the three variables total sv, mean depth and spread in depth varies within these periods. There seem to be a trend of sv to increase into the exposure run as well as after ended exposure, while depth is decreasing in the corresponding periods. Hence, there may be a tendency of a subtle change in schooling density and depth structure over time. For the third variable, spread in depth, no trend could be seen.
Statistical testing showed no significant difference between the different periods (Table 9).

Table 9: *ANOVA results for echosunder data. No comparision of periods was significantly different from each other.*

	p-values		
Comparisons	**Mean depth**	**Depth spread**	**total sv**
Pre-Post	0.9741442	0.9888572	0.5032398
CPA-Post	0.999934	0.9995191	0.9333205
Start-Post	0.9792783	0.8505636	0.7216967
Stop-Post	0.9999137	0.9999704	0.9985366
CPA-Pre	0.9893688	0.998838	0.910168
Start-Pre	0.9999996	0.9827341	0.9953483
Stop-Pre	0.9901059	0.9956686	0.6661505
Start-CPA	0.9920859	0.9288945	0.988412
Stop-CPA	1	0.9999683	0.9861834
Stop-Start	0.9926833	0.8932604	0.8619501

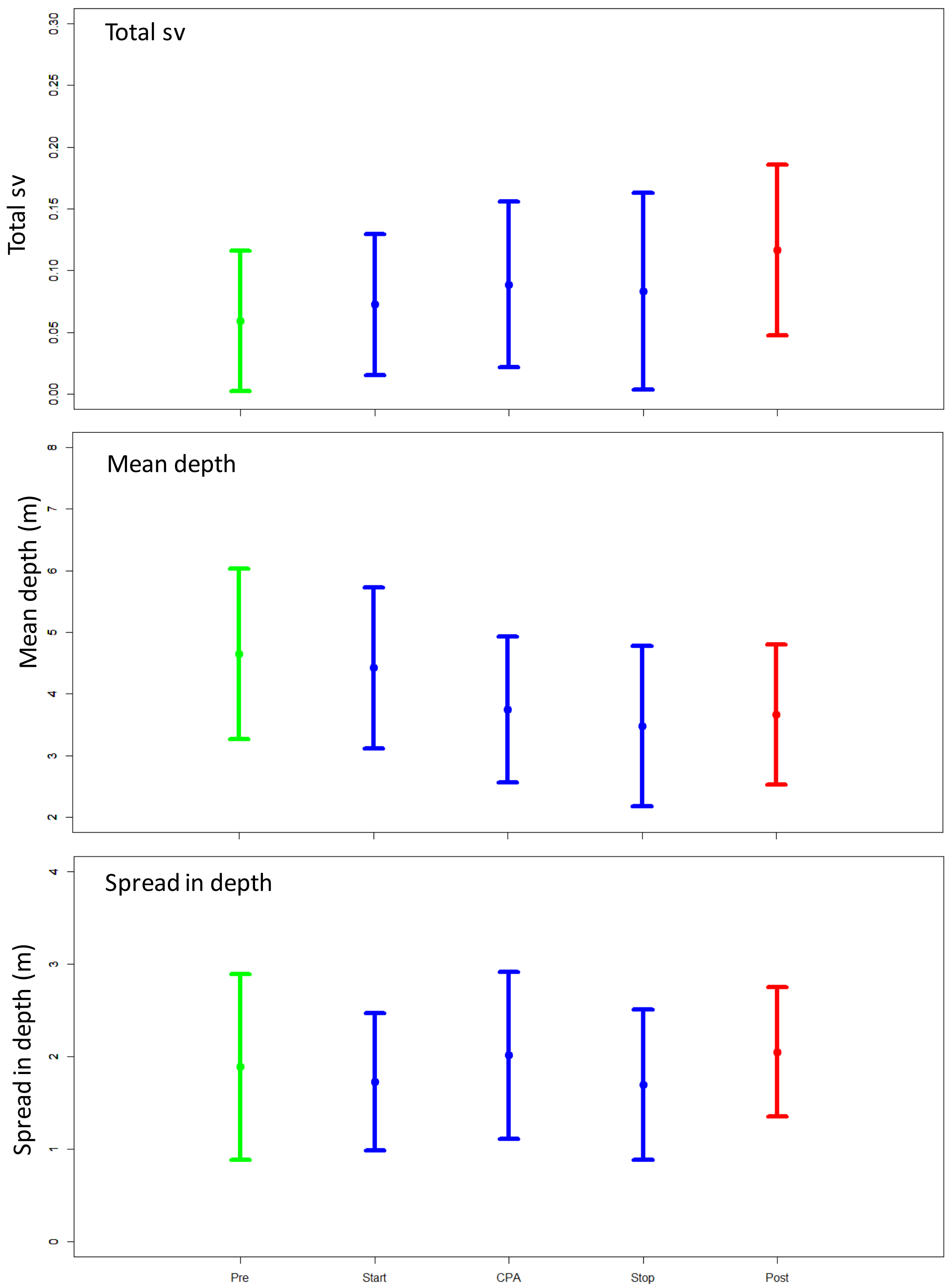

Figure 41. *Mean and standard deviation together for blocks 6,7,9 and 10 for variables total sv (upper panel), mean depth (middle panel) and spread in depth (lower panel). Green colour indicate period before exposure start, blue is during exposure and red is after ended exposure.*

Additionally, we wanted to see how a potential response varied between two succeeding exposures. Figure 42 shows the variations of all three variables for two succeeding blocks of two different batches. Total sv varies for the two blocks within each batch; in general, the second block within each batch has higher sv values compared to the first block for all time periods (Pre, Start, CPA, Stop and Post). For both batches, the Pre phase is higher in the second block compared to the first block, indicating that fish do not return to an initial "undisturbed" swimming dynamic between blocks. Echogram of block 6 (Figure 38), also indicates an higher total sv (greater total density) during CPA. A possible explanation is that fish that were being below the echosounder move up. However it can also be due to the school swimming over the echosounder. Overall, this highlights a change in schooling dynamic over time with fish modifying their schooling behaviour after repeated exposures.

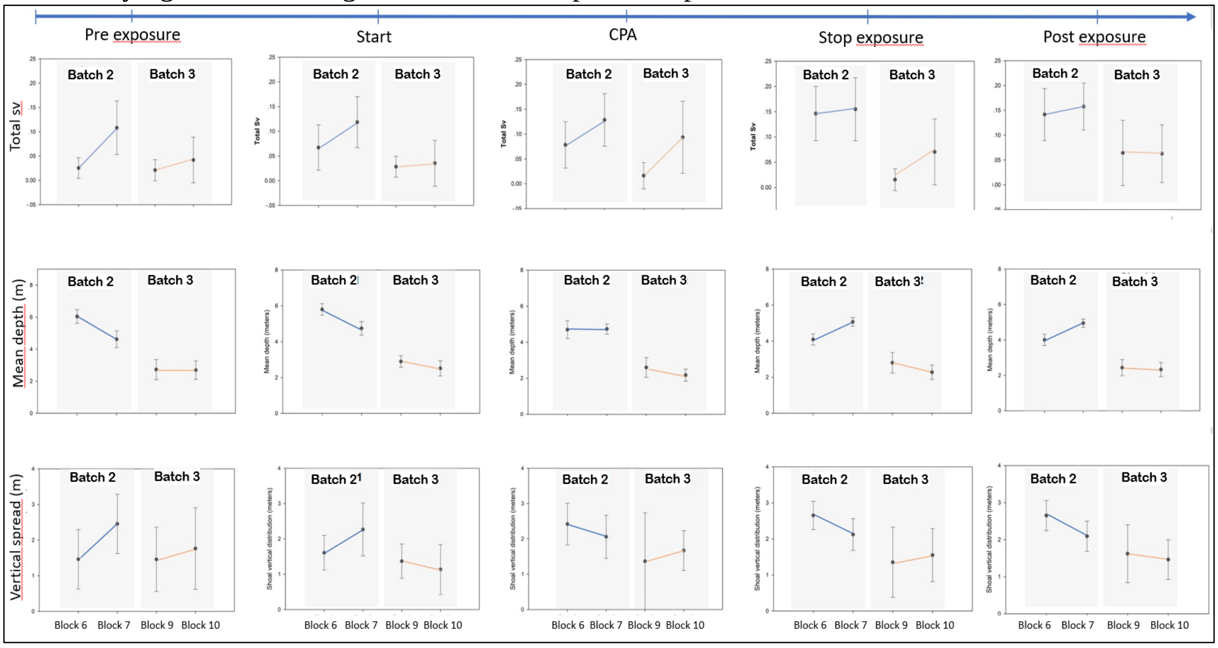

Figure 42. *Total sv (upper panel), mean depth (middle panel) vertical spread (lower panel) for periods before, start, CPA (maximum exposure), end and after for blocks 6 and 7 in batch 2 (blue lines) and blocks 9 and 10 (orange lines) of batch 3. Figure show mean and standard deviation, and each time bin is 3 min long.*

No clear pattern can be inferred for the two others metrics: mean depth and vertical spread.
Mackerels in batch 2 were generally located deeper in the water column in the Pre and Start periods for the second block compared to the first one. At CPA, fish were distributed at similar depth and after swam up to more shallow depth in the second block compared to the first in the Stop and Post periods. For batch 3, the mean depth stayed approximately constant, or slightly deeper in all periods. It is noticeable that the depth prior to any disturbances (Pre periods of block 6 and 9) are different. Batch 3 was distributed in a deeper part of the water column compared to batch 2. It is likely that sound perception may have been different. For Batch 2, a smaller vertical spread was observed for the second block compared to the first one at the CPA, Stop and Post periods. A lower vertical spread indicates that the school is more vertically packed, indicating that the fish collectively reacted to the second exposure by changing their schooling behaviour. However, a similar response patterns is not observed for batch 3, for which, conversely, no change in vertical spread is reported between the two succeeding blocks.
All together, these results show that two batches of mackerel differed in their response patterns to successive blocks of escalating seismic exposure. It is worth noticing that all the individuals tested in our experiment are all of the same age-size class, experiencing thus equivalent fitness tradeoffs, motivational and physiological states. Total number of fish within the batches were also approximately the same (~200 fish). Therefore, it is unlikely that school intrinsic characteristics or individual-based difference may have caused the observed variations in reactions between the batches.

Block 11, seismic still close, was done to provoke a reaction of the fish, and we did observe the fish to swim fast up and down in the net pen in response to this exposure (see Figure 17). Unfortunately, there are several datagaps during this block. However, it can be seen that the mean depth become much more variable after start of run 2, continuing like this throughout the second run as well as after ended exposure (Figure 43, upper level). The same pattern can be seen for vertical spread (Figure 44, lower level), with the spread increasing and becoming far more variable after start of run 2. Run 1 was conducted at further distance, and consisted only of 3 shots, without similar obvious responses.

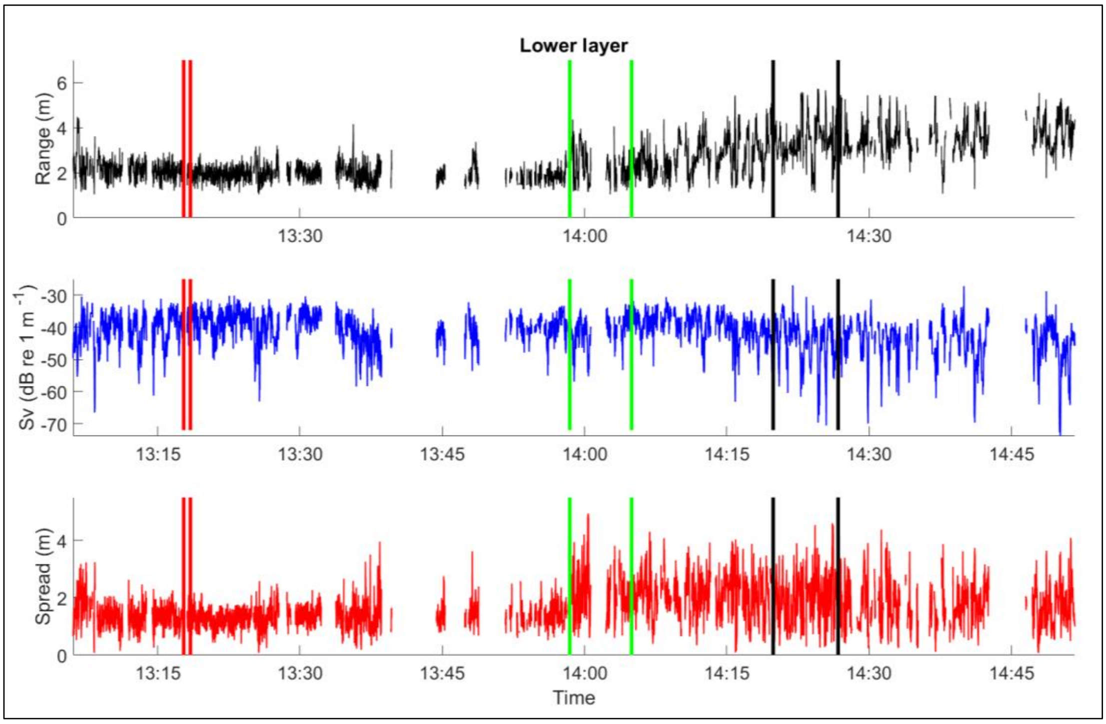

Figure 43. *Time series plot of Block 11with three runs, run 1 (red) are only 3 shots, while runs 2 and 3 (green and black, respectively) are each of 40 shots from the same distance, 90 m. Figure show mean depth (upper level), total sv (middle panel), spread in depth (lower panel).*

4.4 Behavioural scoring

Scoring was divided based on the different sets of introducing the sound; dose escalation, seismic short and seismic still close. In addition, one block with only the source vessel transiting the line without any seismic (control). Figure 44 show the results for all the blocks analysed for dose escalation, while similar plots for seismic close, seismic still and control can be found in Appendix C.

4.4.1 Dose escalation

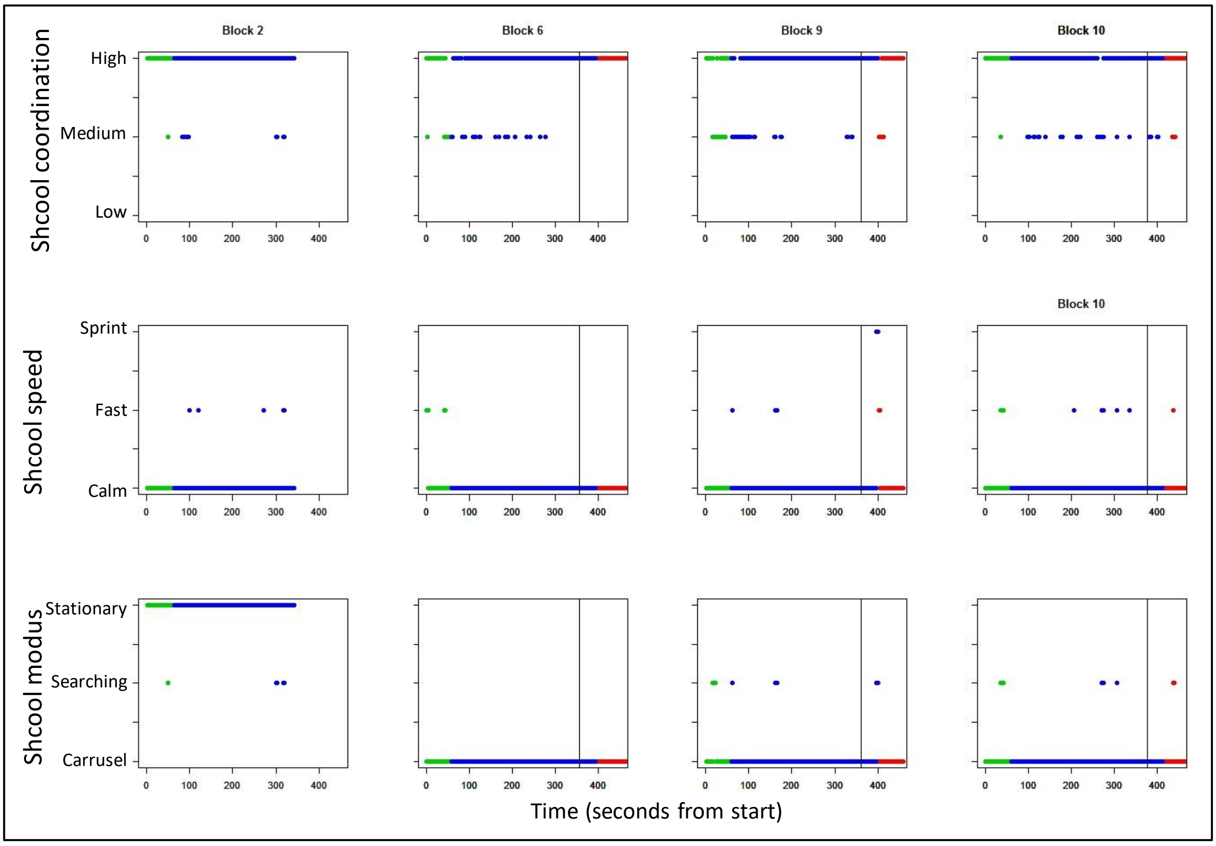

Figure 44. *Time series plots for the four blocks scored with seismic dose escalation, blocks 2, 6, 9 and 10. Green points are the Pre period (before start), blue are during shooting, and red are the post exposure (after ended shooting). Black vertical lines indicate CPA. Note that block 2 lack CPA and post exposure, due to videorecording stopped too early.*

During dose escalation (Figure 44), fish mostly had a school modus of either carousel swimming (blocks 6,9,10) or stationary schooling (block 2), with occasional transitions into search mode, often accompanied by an increase from calm to fast swimming, and a decrease in coordination from high to medium. Such transitions occurred in all three periods (Pre, Dur, Post).

To enable to look more closely into the specific phases of the dose escalation runs, as well as having the same amount of data point for each phase, periods of 5 min was selected as following; 5 min just before the first pulse (Pre), the 5 first minutes of the run (Start), the 5 min surrounding CPA (CPA) and the first 5 min after ended run. The % of time spent in each category for each of these phases are shown in Figure 45. For school coordination (Figure 45, left), time spent as highly coordinated increase from 73% in the Pre phase to 82% in the start phase and further to 93% around CPA, thus indicating a somewhat stronger cohesion of the school during exposure. This is backed up from the statistics, with the ANOVA test showing the CPA period to differ significantly from the Pre and Start periods (Table 10).

For school mode, the fish in blocks 6, 9 and 10 have carousel swimming as the regular mode, while in block 2 stationary schooling is the regular mode. Searching mode is per definition when the fish react, e.g. by swimming up and down and around in the pen. Thus a transition from regular mode to searching should be regarded a reaction. However, the time spent in search mode are almost constant between the phases; 7% in the Pre phase, 6% in the Start, 3% at CPA and 4 % in the Post phase

(Figure 45, middle), thus not giving any indication that such transitions are more common during exposure. The statistic show no significant differences between the phases (Table 8).

School speed is in all phases mostly calm swimming, with occasional transitions into fast swimming, often scored together with transitions to search mode, with time spent as fast swimming being 8, 0, 4 and 3 % of the time in the Pre, Start, CPA and Post phases, respectively (Figure 45, right panel). Hence neither any indication of an increase in school swimming speed during the sound exposure, as also confirmed by no significant differences found between the phases.

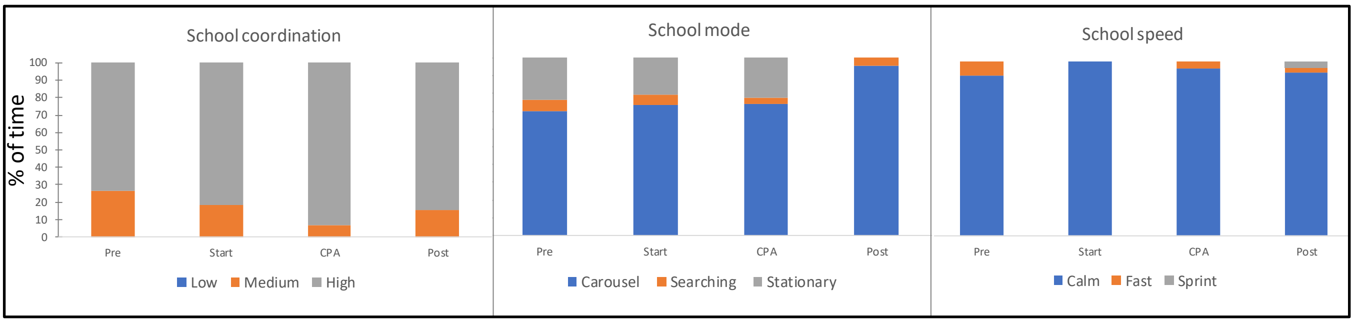

Figure 45. *show the percent of time spent in each scoring category for the phases Pre, Start, CPA and Post for the three different scoring types; School coordination (upper panel), school mode (middle panel) and school peed (lower panel).*

Table 10: *ANOVA results. Table show the p-values comparing the different phases. For scoring "Mode", block 2 are tested separately. Bold p-values indicate a significant difference.*

	p-values			
Comparisons	**Coordination**	**Speed**	**Mode (block 6,9,10)**	**Mode (block 2)**
Post-CPA	0.3443351	0.1345899	0.0963204	0.6046531
Pre-CPA	**0.0007399**	0.4050064	0.0880989	-
Start-CPA	**0.000204**	0.8866824	0.8643177	0.1477863
Pre-Post	0.21918	0.8923141	0.9999534	-
Start-Post	0.1151552	0.026358	0.4198261	-
Start-Pre	0.985397	0.1102918	0.3951085	0.5980358

4.4.2 Seismic close

The two blocks were conducted on different days, and on different batches (see Table 5), and variation seem to be greater between blocks than between the different phases (Pre-Dur-Post). For block 5, school coordination is changing from medium to high after the first run, while for block 8, it is varying between high and medium, with most time spent as highly coordinated.

For school speed, fish in block 5 are swimming calmly at all times, while in block 8 they were swimming fast most of the time, with some transitions to calm. During scoring of block 8 it is noted that there was discussion of whether to score the speed as 1 or 2, but compared to block 6, which were scored before, and conducted on the same batch and same day, the fish appeared to swim faster. This is in line with the results found for swimming speed in the above swimming speed analyses, were a clear trend of increasing swimming speed is seen for each succeeding block for this batch (see figure 38). There is also a clear change in mode for block 8, changing from carousel to searching. This transition start just before the first seismic pulse, thus not likely to be cause by the sound, and last throughout the exposure, before going back to carousel mode in the Post period. This change can also be seen in the echogram for block 8 (see Appendix B), were it can be see that a change occurs around start, but most clear around CPA.

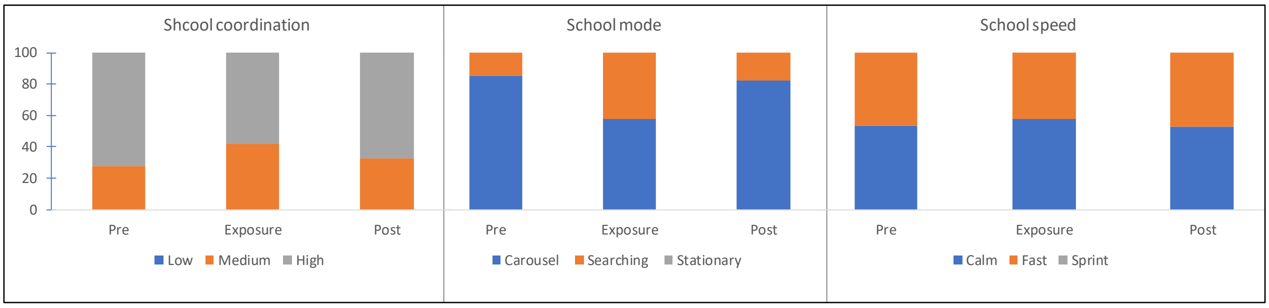

Figure 46. *Seismic close (blocks 5 and 8). show the percent (%) of time spent in each scoring category for the phases Pre, Start, CPA and Post for the three different scoring types; School coordination (left panel), school mode (middle panel) and school speed (right panel).*

Looking at the time spent in each scoring category (Figure 46), more time is spent at high coordination during exposure than before (Pre) and after (Post), and school mode is more often shifting to searching during exposure. For school speed however, no apparent difference between the three periods.

4.4.3 Seismic still close

For the seismic still close (block 11), the mackerel seem to respond to the seismic by shifting from carousel to search mode (Figure 47). This did however occur also in the Pre period, thus likely as the source vessel (Mosby) manoeuvred into position, causing much noise and likely also the water movement from this manoeuvring could have affected the fish. The searching behaviour, by swimming up and down in the net pen, lasted throughout the first run, but went back to carousel swimming for a period, before switching back to search mode, which then lasted throughout the remaining exposure and post exposure phase. The shifts in school mode occur somewhat concurrently with shifts school speed for the first run, but they appear to swim relatively calm for most of the period. Also changes in coordination occur as they shift mode in the first run, but later more varying.

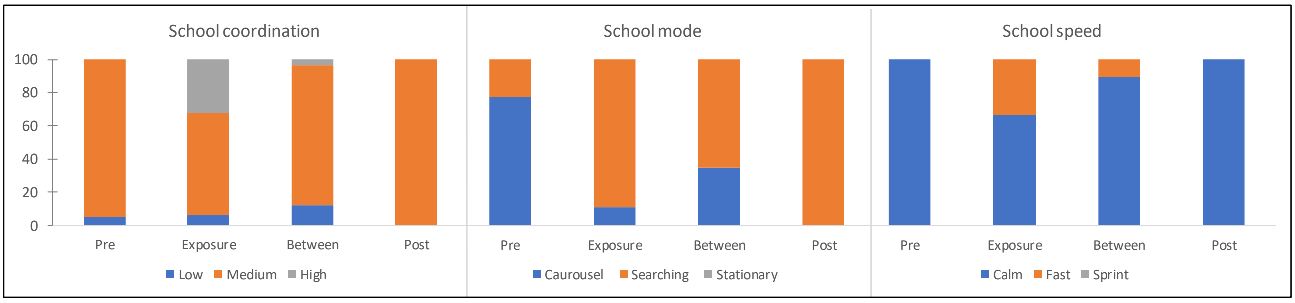

Figure 47. *show the percent (%) of time spent in each scoring category for the phases Pre, Exposure, Between and Post for the three different scoring types; School coordination (left panel), school mode (middle panel) and school peed (rigth panel).*

Looking at the percent of time spent in each phase (Figure 47), it is clear that time spent at high school coordination is greatest during exposure. For school mode, there is an obvious trend that during, between and after exposure, most time is spent in search mode, while school speed is fast a larger fraction of time during and between exposures. Since there are only two runs with this exposure type, the data is too limited for any statistical analysis. However, based the figures presented here, there seem to be a reaction to the seismic exposure when exposed to the levels used here.

4.5 General behaviour

Captive mackerel in this experiment did not show any prominent reactions in terms of swimming speed, diving or schooling dynamics to seismic air guns at received sound pressure SPL from 146 to 171 dB re 1µPa and SEL of 124 to 151 dB re 1µPa^{2}s (bandpassfiltered 5-400 Hz, measured at hydrophone 1 inside pen). The corresponding particle acceleration ranged from 0.02 to maximum of 0.15 ms^{-2}.

The fish did however show reactions by conducting collective responses in terms of rapid swimming up and down in the net pen during incidental waves hitting the net pen, demonstrating the fish`s ability to react as well as the nature of typical collective responses.

All though no sudden responses, both the videoscoring and the total sv (from echosounder) did show an increase in school cohesion from baseline level (Pre) to the time of maximum exposure (CPA), indicating the schooling dynamic to change over time during exposure.

Data from the echosounder and measurement of swimming speed for succeeding blocks on the same batch (blocks 6, 7, 8) of fish indicate that both swimming speed and schooling dynamic seem to slightly change over time, with fish swimming faster and more cohesive for each succeeding block. This may potentially be a response to the repeated exposure.

When the fish was exposed a seismic air gun firing at closer range (90 m), thus increasing the received SPL to 184 dB re 1µPa and SEL to 160 dB re 1µPa^{2}s (bandpassfiltered 5-400 Hz, measured at hydrophone 1 inside pen), reactions in terms of changes in school mode, from carousel swimming to searching behaviour. The mackerel was than seen to swim up and down the net pen at increased speed. However, this behaviour was also seen as the source ship backed towards the pen to get into position.

4.6 Measurements at aquaculture farms

The IMR scientist who monitored the videos from the fish farm live during experiments, did not report any unusual behaviour, and no stress or abnormal behaviour. Employees from the fish farm, watching together with the scientist, agreed on this, and did nor see any form of unusual behaviour. was obvious during constant monitoring during the experiments. All videos were later carefully scrutinized visually, one by one. The number of visual observations, both at the fish farm and later form the videos, are rather high and in none of them show any response among the fish. Table 11 list the number of net pens observed

Table 11: *Results from visual observations at fish farms and videos Table indicate the number of net pens observed and number of responses noted during the observations done in real time at the fish farms during exposure, as well as number of net pens and responses during the later scrutinizing of videos.*

Skorpo (Rainbow trout)	**Observations at fish farm**	**Visual scrutinizing of video**
Response start seismic	non in 18 of 18 observed pens	non in 18 of 18 observed pens
Response at CPA	non in 16 of 16 observed pens	non in 16 of 16 observed pens
Response after CPA	non in 17 of 17 observed pens	non in 17 of 17 observed pens

Kyrholmen (Salmon)	**Observations at fish farm**	**Visual scrutinizing of video**
Response start seismic	non in 12 of 12 observed pens	non in 7 of 7 observed pens
Response at CPA	non in 12 of 12 observed pens	non in 7 of 7 observed pens
Response after CPA	non in 12 of 12 observed pens	non in 12 of 12 observed pens

Flatøyflu (Salmon)	**Observations at fish farm**	**Visual scrutinizing of video**
Response start seismic	non in 12 of 12 observed pens	non in 9 of 9 observed pens
Response at CPA	non in 12 of 12 observed pens	non in 8 of 8 observed pens
Response after CPA	non in 12 of 12 observed pens	non in 8 of 8 observed pens

There was work going on in one cage with Rainbow trout and one with salmon, Table 12. In some of the cages at Skorpo there was trouble with the video cameras. One did not work at all and other of them falling out. This is listed as “Cam.error” in Table 12. In some cases, the recording unit used did not work, or did not manage to record video image from all the cages, this is listed as “No rec.” in the tables. The block number and date refers to Table 11 and the observations are all done during seismic dose escalation.

Table 12: *Detailed results from visual video scrutinizing.*

Skorpo (Rainbow trout) CPA approx. 1nmi

Cage nr:	1	2	3	4	5	6
Block 2, run 1 (22/11)						
Camera depth:		39-40m		35-36m		34m
Response start seismic	Cam.error	non	work	non	non	non
Response at CPA	Cam.error	non	work	non	non	non
Response after CPA	Cam.error	non	work	non	non	non
Block 3, run 1 (22/11)						
Camera depth:		40m	34-35m	37m		34m
Response start seismic	Cam.error	non	non	non	non	non
Response at CPA	Cam.error	non	Cam.error	non	non	non
Response after CPA	Cam.error	non	Cam.error	non	non	non
Block 6, run 1 (24/11)						
Camera depth:		33m		27-28m		29m
Response start seismic	Cam.error	non	Cam.error	non	non	non
Response at CPA	Cam.error	non	Cam.error	non	non	non
Response after CPA	Cam.error	non	Cam.error	non	non	non
Block 7, run 1 (24/11)						
Camera depth:		36m	36-37m	27m		29m
Response start seismic	Cam.error	non	non	non	non	non
Response at CPA	Cam.error	Cam.error	non	non	non	non
Response after CPA	Cam.error	non	non	non	non	non

Kyrholmen (Salmon) CPA approx. 1.5 nmi

Cage nr:	1	2	3	4	5	6
Block 9, run 1 (25/11)						
Camera depth:	5m	9m	19m	5m	4m	
Response start seismic	No rec.	No rec.	non	No rec.	No rec.	No rec.
Response at CPA	No rec.	No rec.	non	No rec.	No rec.	No rec.
Response after CPA	non	non	non	non	non	non
Block 10, run 1 (25/11)						
Camera depth	6m	10m	17-18m	9m	8m	
Response start seismic	non	non	non	non	non	non
Response at CPA	non	non	non	non	non	non
Response after CPA	non	non	non	non	non	non

Flatøyflu (Salmon)	CPA approx. 1 nmi					
Cage nr:	1	2	3	4	5	6
Block 9, run 1 (25/11)						
Camera depth:			27m	18m	8m	19m
Response start seismic	No rec.	No rec.	non	non	non	non
Response at CPA	No rec.	No rec.	No rec.	non	non	non
Response after CPA	No rec.	No rec.	No rec.	non	non	non
Block 10, run 1 (25/11)						
Camera depth		26m	3m	18m	8m	19m
Response start seismic	No rec.	non	non	non	non	non
Response at CPA	No rec.	non	non	non	non	non
Response after CPA	No rec.	non	non	non	non	non

5 Summary and discussion

The main findings of this project are summarised in a list of bullet points, and discussed in detail further below.

- Schools of penned mackerel (three batches) were exposed to impulsive sounds from a 90 cubic inch seismic (airgun) source towed behind a research vessel, in a dose-escalation design. Essentially simulating a seismic survey, sound pulses were presented with 10 s repetition rate, with increasingly high received levels, as the proximity of the source vessel was decreased from approximately 7000 to 300 m. Received sound pressure levels (RL) and sound exposure levels (SEL) ranged from 146 to 171 dB re 1µPa and 123 - 149 dB re 1µPa^{2}s, respectively. Sound particle acceleration, the relevant stimuli of the fish inner ear, was measured in the range 0.02 - 0.15 m/s^2 (zero-peak amplitude), and corresponding acceleration exposure levels (AEL) 62 - 80 dB re 1µm^2/s^3, at the fish pen
- Expert elicitation of schooling behavior, based on the video recordings inside the pen, demonstrated an absence of abrupt avoidance behavior and startle responses in the school, i.e. no distinct threshold of avoidance could be discriminated. The visual assessments did however indicate subtle behavioural changes, observed as a gradual increase in school coordination, which culminated around the time of closest point of approach (CPA).
- Analyses of data from a bottom-mounted echosounder also indicate a change during the course of the exposures, seeing as the measure of school density (sv) was generally higher during CPA than in baseline periods. There was also a tendency of higher sv in the second of two repeated blocks of dose-escalation trials, indicating possible sensitization.
- During a shorter and closer exposure run, also exposing the fish to a gradual increase of sound, from 168 to 178 dB re 1µPa (bandpassfiltered 5-400 Hz), similar results of no strong, sudden responses were documented.
- When exposed to seismic air gun pulses at close range (90 m) at received SPL of 184 dB re 1µPa (bandpassfiltered 5-400 Hz), the fish respond by stating to swim rapidly up and down in the net pen. This may be the closest to a vertical response as one can get in a net pen. This clear and sudden reaction could serve as a positive control, and no similar response were found in response to the seismic pulses during the dose-escalation or seismic short trials.
- The present results thus indicate that the threshold between small subtle reactions to clear avoidance responses lies within the range of SPL of 178 to 184 dB re 1µPa, but is likely also influenced by the presentation of the signal, with sudden, loud signals being experienced as more scary than a gradual increase in sound level.
- Strong reactions in terms of rapid swimming up and down the pen in response to incoming sea waves and to the seismic still close exposure demonstrate that the fish are able to show a reaction, as well as for our observation systems to capture such a reaction. The lack of reaction is hence not a lack of the fish`s ability to perform a reaction.
- Neither salmon or rainbow trout at close by fish farms showed any behavioural response as the seismic source vessel passed by, with the closed distance between the vessel and fish farm of about1- 1.5 nautical mile. No sound measurement was done here.

5.1 Comparison with earlier results

In this study, we exposed mackerel to seismic air guns of increasing levels with maximum SPL at ~178 dB re 1µPa and particle acceleration of maximum 0.15 ms^{-2} without any prominent reactions in terms of swimming speed, schooling behaviour or diving. In a previous study (Sivle et al., 2016), exposure to a 14 Hz pure tone at SEL levels of 143 dB re1µPa^2 s and 0.03 ms^{-2} we documented short-term avoidance behaviour (including startle responses) and significant increase in swimming speed. The maximum exposure level was hence remarkably higher during the exposure to seismic air gun, both in terms of sound pressure and particle motion, but without triggering such strong reactions as was found in response to the 14 Hz tone. The differences may be caused by several factors. The most obvious reason is the different way sounds are introduced. In Sivle et al. (2016), fish was exposed to sounds at the maximum level without any form of gradual increase, while in 2016, fish experienced a gradual increase in sound levels from almost audible at the first shoot, with regular shoots every 10 sec for almost 1 h before the maximum level was reached. It is therefore possible that the gradual increase made the maximum level less scary. This may also be supported by the reactions seen for block 11, with clear responses to a sudden seismic pulse at maximum level from the first pulse. Similar results, that sudden sounds are more likely to trigger a response, have also been found for other fish species, e.g. herring (Schwatz & Greer 1984).

Another possibility is the differences in frequency content. In Sivle et al. (2016), strong reactions were found to a 14 Hz pure tone with 3 sec duration. The seismic signal does include this frequency, but the total energy is distributed over a far wider frequency band (see e.g. Figure 28), thus the energy within the lowest frequencies are likely lower than those measured for the 14 Hz. It is well known that many species of fish tend to be highly sensitive to infrasound (Knudsen et al. 1992;1997, Karlsen et al., 2004). It can therefore have been the frequency content that causes the difference in reaction.

A third possibility is the signal to noise ratio. The Sivle et al. (2016) experiments were done in a protected bay, with likely far less background noise. On the contrary, the current experiments were done in a more open fjord, and with the sound source being a noise vessel, thus probably with a far higher background noise than the previous experiment that induce a strong reaction.

5.1.1 Swimming speed

No apparent change in swimming throughout the dose escalation experiment was found in the present study. In the Sivle et al. (2016) study, swimming speeds were then obtained by manual tracking of fish movements in a similar fashion as in the present study, although by means of a different camera placement; i.e. the GoPro was bottom mounted and pointing up, capturing the ventral side of the fish, while in the present study, the GoPro was placed on the net pen wall, thus capturing fish sideways. However, this should not influence the results remarkably, as well as they are both compared to their own baseline. In response to the 14 Hz tone of Sivle et al. (2016), the maximum swimming speed was 2.3 and mean 1.04 m/s during the 5 sec exposure, while the baseline level was 0.48 m/s. This lies well above the preferred swimming speed range of mackerel (Table 10, from Wardle & He, 1988). In the current study, all of the measured swimming speeds falls within the range of preferred swimming speed (0.29 – 1.15 m/s), described by Wardle and He (1988) (Table 13), as well as below baseline levels found in the previous mackerel study of 0.48 m/s (Sivle et al. 2016). The current study does certainly not show any indications of burst swimming < 4.0 m/s, as reported in Wardle & He (1988), which would have indicated that the mackerel were severely startled by the airgun sounds.

Table 13: *Swimming speeds of mackerel recorded in a 10 m long tank, from the study of Wardle and He (1983). Fish lengths of was in the range 0.275-0.380 m. Swimming speeds were mostly reported in body lengths/s, and converted to m/s in this report, based on a mean body length, 0.328.*

Mackerel swimming speeds in Wardle and He (1988)	**body lengths/s**	**m/s**
Maximum speed	18	5.5
Maximum sustained speed over 200 min	3.5	1.15
Minimum speed to in order to maintain constant depth	0.4	0.13
Preferred swimming speed, max	0.9	0.29
Preferred swimming speed, min	3.5	1.15

Results from the swimming speed analysis are in agreement with the expert video scoring of speed, neither showing any substantial change in the general swimming speed of the entire school throughout the exposure period.

5.1.2 Schooling dynamic

Both videoscoring and echosounder data show a tendency that the mackerel form denser schools and are more coordinated during maximum exposure compared to before exposure start. This subtle reaction seems to occur over time and are not directly visible, but indicate that seismic exposure may influence the schooling dynamic of mackerel. A similar response was also documented in Sivle et al. (2016) reporting the fish to align and swim more coordinated after exposure. A comparison of succeeding exposures on the same batch also indicate this reaction to be more pronounced for the second exposure, potentially indicating a sensitization of the school to the sound. As for schooling mode, in most blocks, fish were swimming around in a typical carousel, with occasional transitions into search mode. However, such transitions was generally not long lasting, and did not occur more often during exposure than before exposure started. During the exposure to close, sudden and high level seismic (block 11, seismic still close), there is a clear shift in schooling dynamic with respect to increased coordination, higher schooling speed and shifts from carousel to searching school mode. The two exposure regimes thus result in the same response, but much weaker and spread over longer time during the gradual and lower level dose escalation.

5.1.3 Vertical movement and spread

For seismic dose escalation experiments, we do not find any particular indications that the fish change their depth or become more or less spread out vertically in response to the seismic.
During the seismic close exposure (Block 11), a clear pattern of vertical movement, up and down in the pen were seen, causing a large variation in both mean depth and vertical spread. These reactions were also verified by video monitoring. The rapid swimming up and down the net pen may be the closest as we will get to a form of vertical response, and without the limitations of the net pen walls the fish may have moved deeper than here recorded, and not back up to the surface as they did here when meeting the net pen floor. This pattern can thus be identified as how a clear response will look like. The absence of any systematic reaction of this type during the dose escalation hence strongly suggest that the seismic pulses presented at these level and with this gradual increase, does not induce any strong vertical avoidance responses.

5.1.4 Potential habituation or sensitization

In addition to maximize the amount of data, one reason for conducting multiple runs on the same batch was to identify potential habituation (weaker reaction with repeated exposure) or sensitisation (stronger responses with repeated exposure). Data on swimming speed suggest an increased swimming speed throughout one day with succeeding exposure, suggesting a subtle response to repeated exposure. For this same day (block 6, 7 8), also the echosounder data suggest a change in the distribution and schooling dynamic over time. However, with the limited amount of data replicates as well as control e.g. for a day without any exposure, we cannot conclude anything.

5.1.5 Exposure to salmon and rainbow trout at fish farms

The frequency range of seismic sounds are within the main hearing range of salmon as defined by Hawkins and Johnstone (1978), and salmonid fish have been documented earlier to show flight responses to infrasound (<20 Hz) (Knudsen et al. 1997), thus representing the lowest frequency spectre of the seismic. However, in this study it was not possible to visual detect any responses among either salmon or rainbow trout, not even by carefully analyse the video material. Unfortunately, we did not have available any hydrophones to measure the sound pressure at the fish farms, and thus do not know the received sound pressure level experienced by these fish.

All fish farms in the area was contacted in advance of the experiment, and the experiment and its purpose carefully explained. As all farms in the area had the same owner (Lerøy Vest AS). We had a close dialog and gave them the opportunity to choose between several different transects and to give input to us which would be the best option regarding causing less disturbance.

We experienced the dialogue as very constructive, and think that being open and explaining the work was very useful and gave us an opportunity to obtain some indications of how farmed salmon may respond to seismic sounds.

5.1.6 What could have been done differently?

One of the main objectives of this study was to investigate how exposure to seismic air gun affected feeding motivation of mackerel. Seismic exposure has been shown to decrease the feeding motivation of other fish species such as haddock (*Melanogrammus aeglefinus*), Greenland halibut (*Reinhardtius hippoglossoides*) (Løkkeborg et al. 2012) and rockfish (*Sebastes sp*.) (Skalski et al. 1992). Due to an extensive mackerel bait fishery coinciding in time and place with regular seismic activity, it is important to understand how their motivation to respond to baited hooks may change during seismic exposure, in order to manage seismic activity and fishery in a good way. Unfortunately, we were not able to do any such measures, do to the fact that the fish basically were not interested in the food pellets at all. The experiment was done in late November, with sea temperature below the ideal temperature for mackerel. The fish was captured in this fjord during summer and kept in net pens at Austevoll station since then. They had a good appetite, feeding on fish pellets, throughout the summer and most of the autumn, but in the weeks prior to the experiment, the appetite was decreasing, and at the time of the experiment, they only fed occasionally on the pellets offered. Therefore, it was not giving any good way of measuring how appetite changed with exposure to seismic air guns.

Ideally, this entire experiment should have been done during the summer, when this fjord is a natural seasonal habitat for mackerel, their appetite is good, and also more daylight would have made our measurements much better and extended the amount of workable hours every day. However, as the project was initiated in July, and had to be finalized within the same year, the first possible option to get all the logistics needed; vessels, air gun, people, measuring equipment was November.

5.1.7 Application of results

These studies were done on fish in captivity, in a non-optimal season, with a far lower air gun source than are used in conventional seismic surveys. The results should hence therefore not be directly applied to free living mackerel in other seasons. However, these results do indicate that mackerel does not show severe reactions to seismic exposure, in particular when exposure in gradually increasing. As seen with the reactions to waves etc. these fish does seem more reactive when the exposure in sudden and extensive. Experiments of similar behavioural traits of mackerel should be done in the field with free ranging fish to better be able to enable a generalization of their behavior to seismic air gun exposure.

The finding that mackerel seem to habituate to the gradual increase in sound level resulting in them not to show any abrupt reactions compared to when sound is introduced suddenly and at high levels is also an important finding that can be applied in a real seismic operation, e.g. that all seismic operations in areas with likely mackerel encounter should use ramp up procedures before going to full power surveying.

One of the aims of the study was to evaluate how observed responses may affect the fishery. Typical reactions that will reduce catchability are sudden rapid movement such as diving or increased swimming speed. Our results indicate that such responses are more likely induced by a sudden introduction of a loud sound than by a gradual increase in sound level. A sudden vertical or horizontal avoidance is thus more likely close to the source vessel at start up. However, it must be taken into consideration that the mackerel was captive, thus with no real horizontal escape ability, and limited vertical escape ability, as well as using a downscaled single air gun compared to the large air gun arrays used in typical seismic surveys.

Measurements of sound level at the aquaculture facilities will have increased the value of this part of the experiment. However, the visual observations are sufficient as an initial study.

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7 Appendixes

7.1 Appendix A: Additional hydrophone results

Block 6

Measurements were made at two depths inside the net pen. The peak sound level for both hydrophones are plotted versus pulse number in Figure A1, both for unfiltered and filtered signals. The deepest hydrophone gives the highest peak values. Therefore, for the rest of the results only plots from hydrophone 1 at 5 meters are presented.

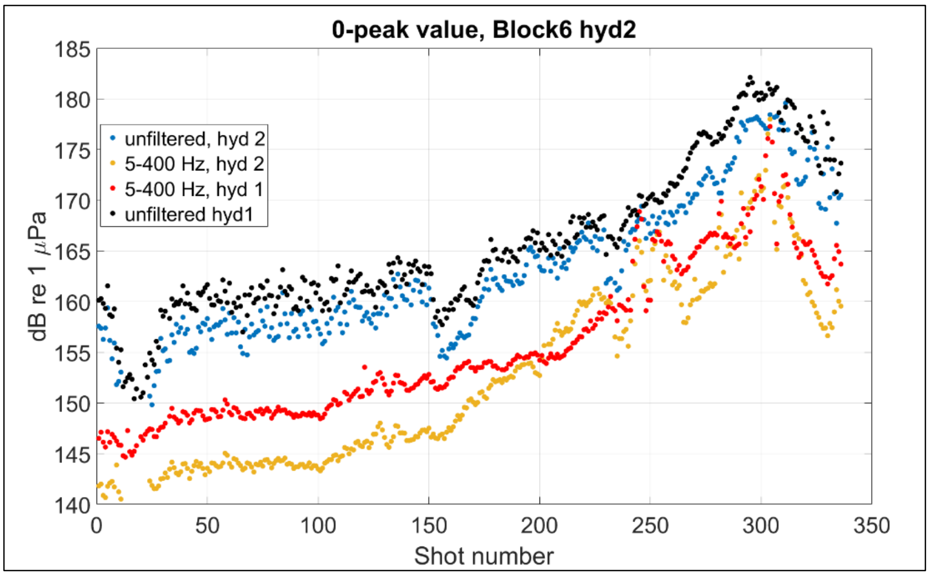

Figure A1. *Compare hydrophones: Peak amplitude measured with the two hydrophones. Hyd 1 at 5 m and hyd 2 at 3 m depth. The figure shows filtered and unfiltered results. The measurements at 5 m have higher amplitude than those measured at 3 m.*

The frequency content in pulses at different distances is shown in Figure A2. One second long sequences of signal around the seismic pulses are analyzed. Similar noise-sequences are selected about 2 seconds before the seismic pulse. The frequency content of the noise and of the seismic pulse is compared in Figure A2. It can be seen that pulse number 1, at 7 km distance, is quite broad banded with most of the energy between 300 and 1600 Hz. This is the same for pulse 188 which was measured at a distance about halfway between 7 km and 330 m, thus around 3335 m from the net pen. The pulse at CPA (330 m distance) has much higher amplitude than the two other pulses, and the frequency distribution differs from the two prior examples. The pulse at CPA has most of its energy between 10-200 Hz, which is in the center of fish hearing. The pulse at CPA has also higher amplitude for the frequency range 200-2000 Hz than pulse number 1 and 188. This is demonstrated in the upper panel of Figure A3 where the frequency response from the three pulses at different distances are plotted on top of each other. The panel in the middle, and the lower panel of Figure 27 shows the Energy Spectral Density for the three pulses compared to each other, first with frequency steps/bands of 1 Hz, then with 1/3 octave bands in the bottom panel. The dB scale makes the differences in amplitude look smaller. The ESD results differs the most for the lowest frequencies.

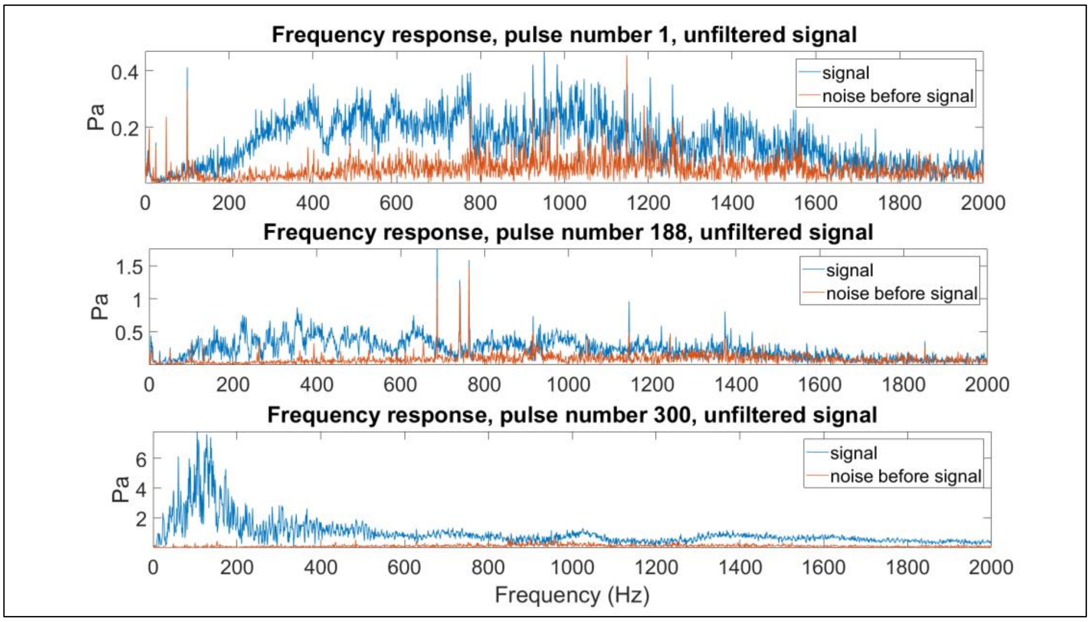

Figure A2. *Frequency content of pulses at different distances from the seismic source. Pulse 1: 7000 m, pulse 188: half way, pulse 300: 330 m. Note the different scale in sound pressure between the subplots.*

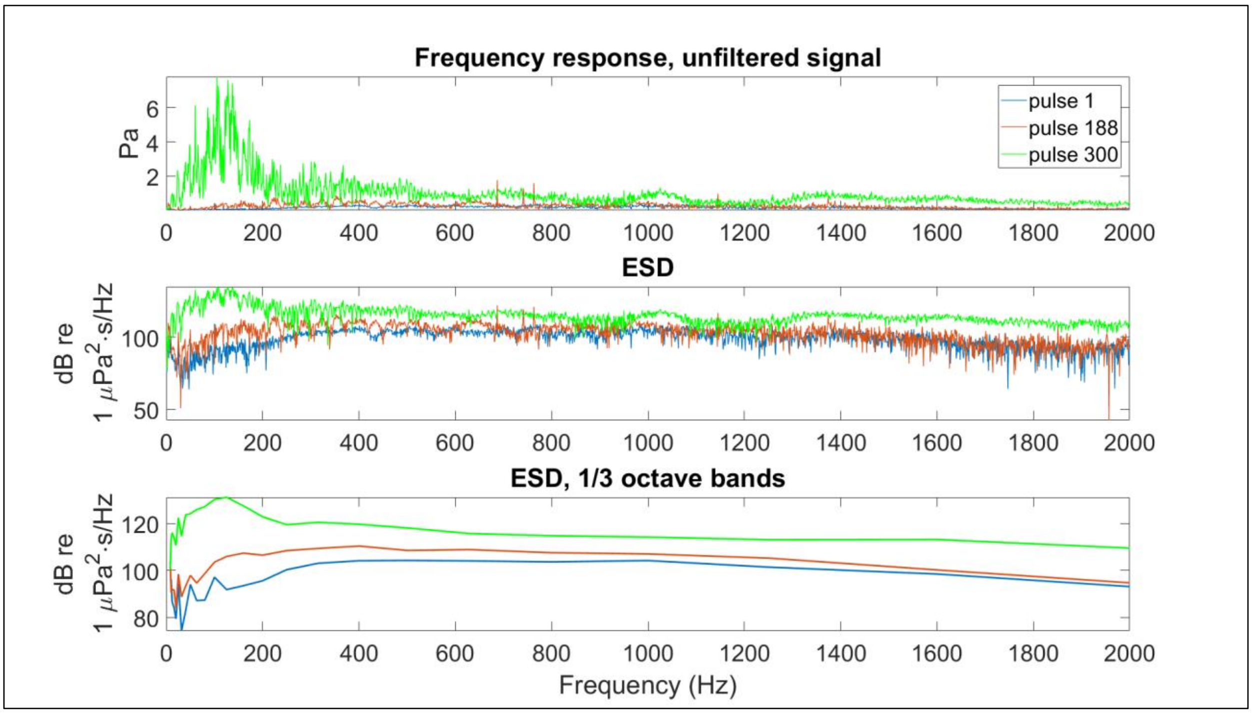

Figure A3. *Compare frequency response and ESD for 3 pulses at different distances. Pulse 1: 7000 m, Pulse 300: 330 m.*

Block 8

Figure A4 shows the recordings with hydrophone 1 from block 8. The noise level of the ship, Mosby, can be seen from about time 14:20 in the figure.

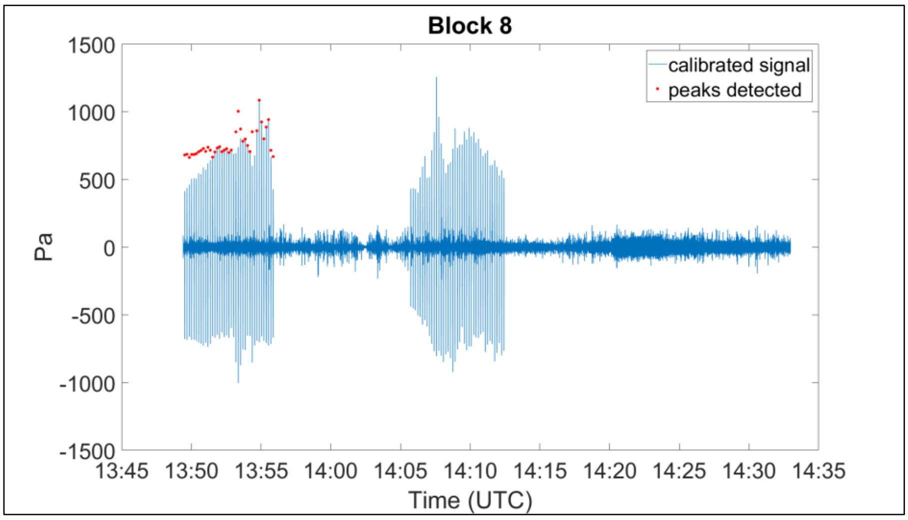

Figure A4. Block 8, hydrophone 1 at 5 meter depth. 3 runs. The two first with 40 seismic shots each, the last run is without seismic, only the boat noise from time 14:20. Time is given as UTC.

Block 11

Figure A5 shows all the 40 shots which was transmitted from the same distance. The positive peak is higher than for the negative peak for all the shots. The positive peak is higher than for the negative peak for all the shots. This is the opposite of the CPA-pulses from Block 6 and 8. The amplitudes are around 3200 Pa or 190 dB for the unfiltered results. This is about 10 dB higher than at CPA for the other transect types.

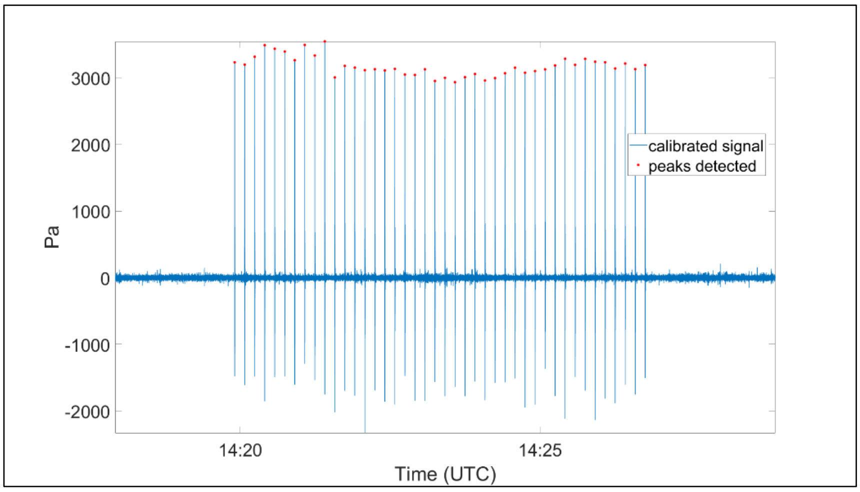

Figure A5. *Block 1, run 3, hydrophone 1 at 5 meter depth, 40 seismic shots. Time is given as UTC.*

7.2 Appendix B: Additional echosounder results

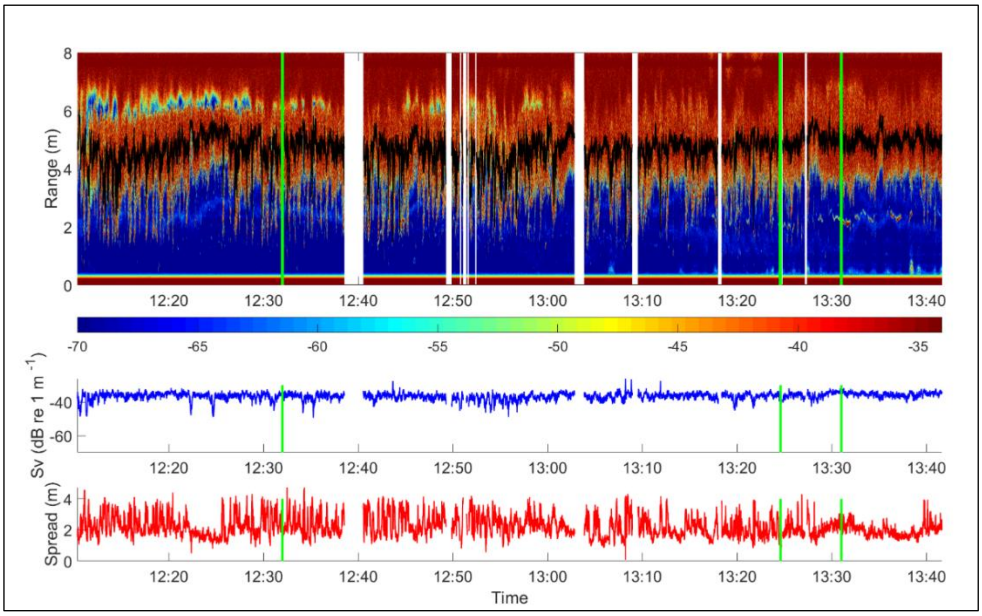

Figure B1. *Block 7: This is the continuation of Block 6 (**Feil! Fant ikke referansekilden.**). Seismic exposure for Block 7 started about 1 hour and 20 minutes after end of Block 6. Mean depth is plotted as a black line on top of the echogram (upper level), the total sv (blue line) (middle panel), and the depth spread (lower panel). Start, max and stop of seismic shooting is marked with green vertical lines. White gap indicate missing data. "Range" in the echogram indicate the distance from echosounder, placed at approximately 8 m depth. The thick red colour at range 7-8 on top of the picture is thus the sea surface, while range=0 is about 8 m deep. Time is given as UTC:*

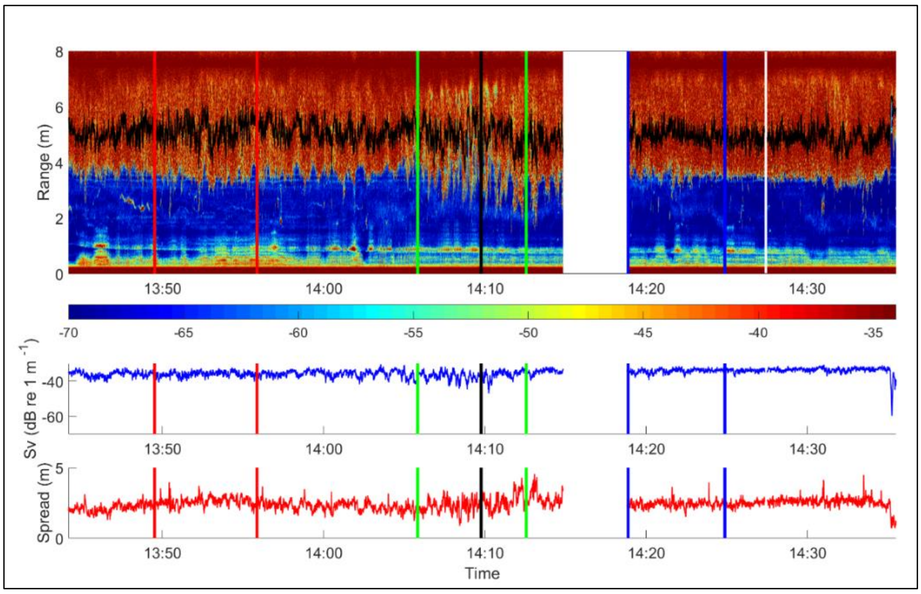

Figure B2. *Block 8: three short runs with 40 shots each for the two first. The third run was a control with only the boat and no seismic. Run 1 is marked with red, run 2 is marked with green, run 3 is marked with blue. CPA is marked with black. Otherwise, all panels are similar as figure B1.*

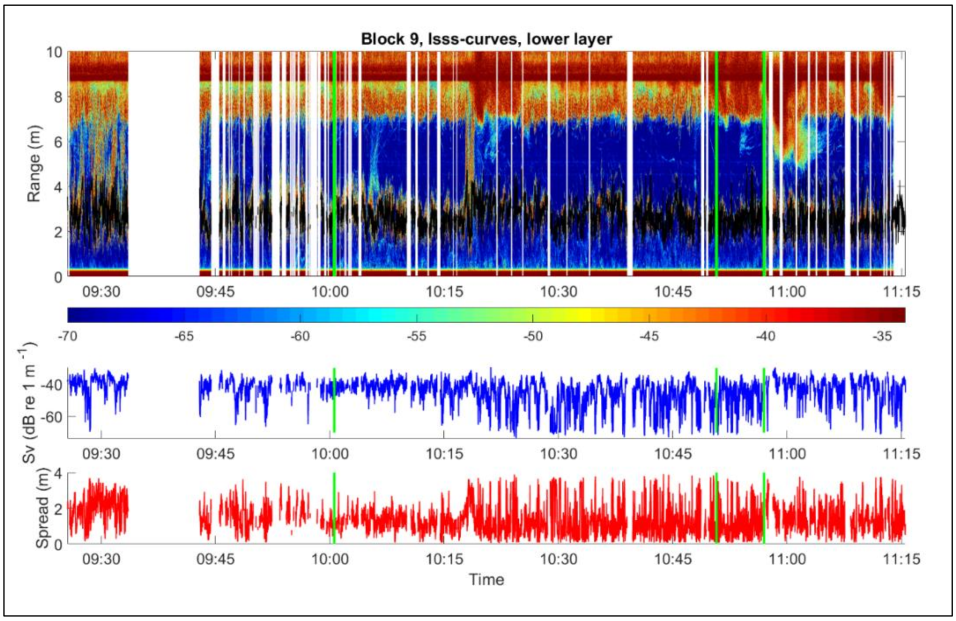

Figure B3. *Block 9, two layers, but only the lower layer was analyzed. Mean depth is plotted as a black line on top of the echogram (upper level), the total sv (blue line) (middle panel), and the depth spread (lower panel). Start, max and stop of seismic shooting is marked with green vertical lines. White gap indicates missing data. "Range" in the echogram indicate the distance from echosounder, placed at approximately 8 m depth. The thick red color at range 7-8 on top of the picture is thus the sea surface, while range=0 is about 8 m deep. Time is given as UTC.*

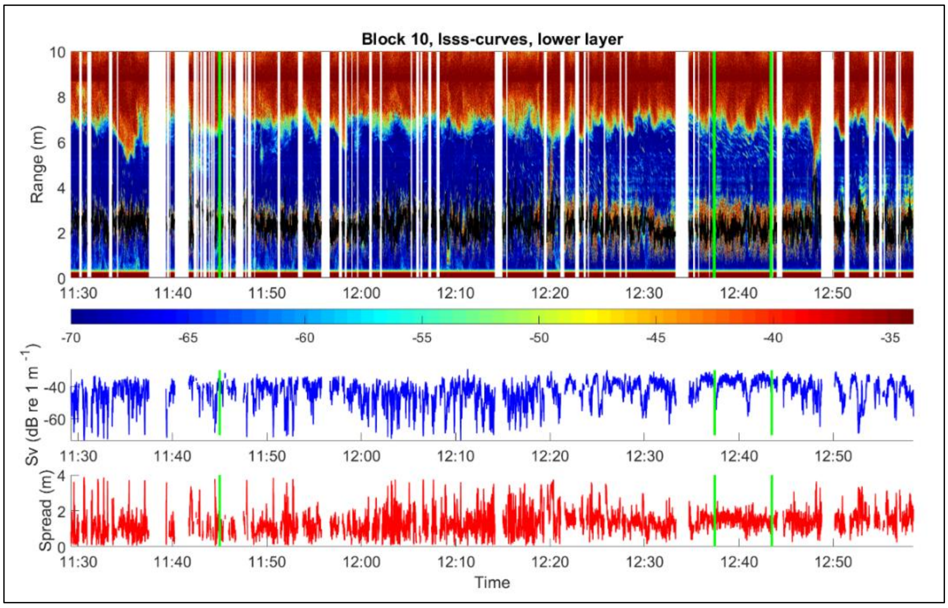

Figure B4. *Block 10 two layers, but only the lower layer was analysed. Mean depth is plotted as a black line on top of the echogram (upper level), the total sv (blue line) (middle panel), and the depth spread (lower panel). Start, max and stop of seismic shooting is marked with green vertical lines. White gap indicate missing data. "Range" in the echogram indicate the distance from echosounder, placed at approximately 8 m depth. The thick red colour at range 7-8 on top of the picture is thus the sea surface, while range=0 is about 8 m deep. Time is given as UTC:*

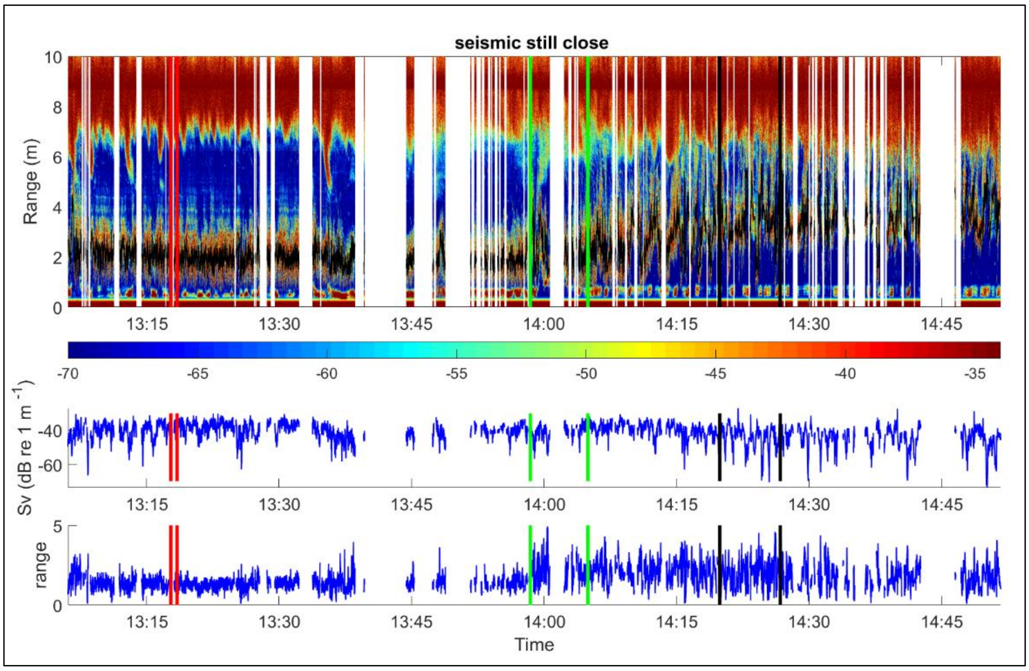

Figure B5. *Block 11, with 3 runs. Run 1 (red) are only 3 shots, while runs 2 and 3 (green and black, respectively) are each of 40 shots from the same distance, 90 m. Mean depth is plotted as a black line on top of the echogram (upper level), the total sv (blue line) (middle panel), and the depth spread (lower panel). Start, max and stop of seismic shooting is marked with green vertical lines. White gap indicate missing data. "Range" in the echogram indicate the distance from echosounder, placed at approximately 8 m depth. The thick red colour at range 7-8 on top of the picture is thus the sea surface, while range=0 is about 8 m deep. Time is given as UTC.*

7.3 Appendix C: Additional results from video scoring

Seismic short

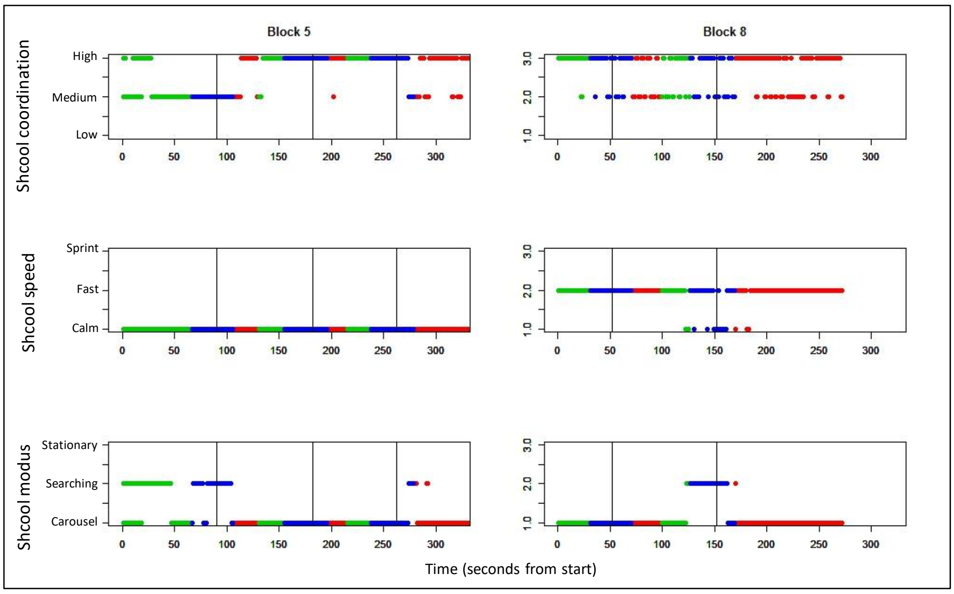

Figure C1. *Time series plots for the two blocks scored with seismic short, blocks 5 and 8, with 3 and 2 runs included, respectively. Green points are the Pre period (before start), blue are during shooting and red are the post exposure (after ended shooting). Black vertical lines indicate CPA.*

Seismic still

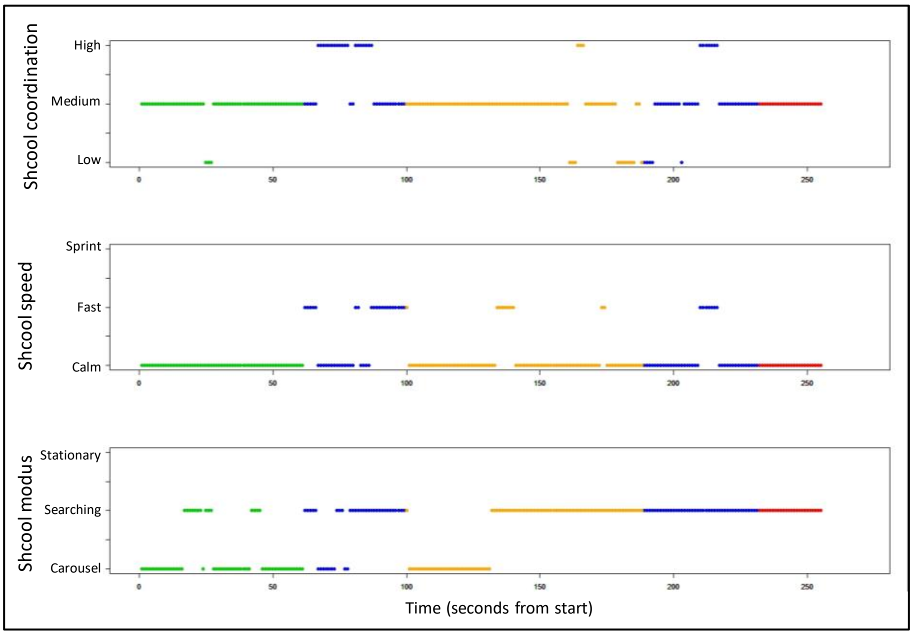

Figure C2. *Time series plots for the one block with seismic still, block 11, including 2 runs. Green points are the Pre period (before start), blue are during shooting, orange are between succeeding runs and red are the post exposure (after ended shooting). As the source ship does not move, no CPA is given as the sound level is constant.*

Control

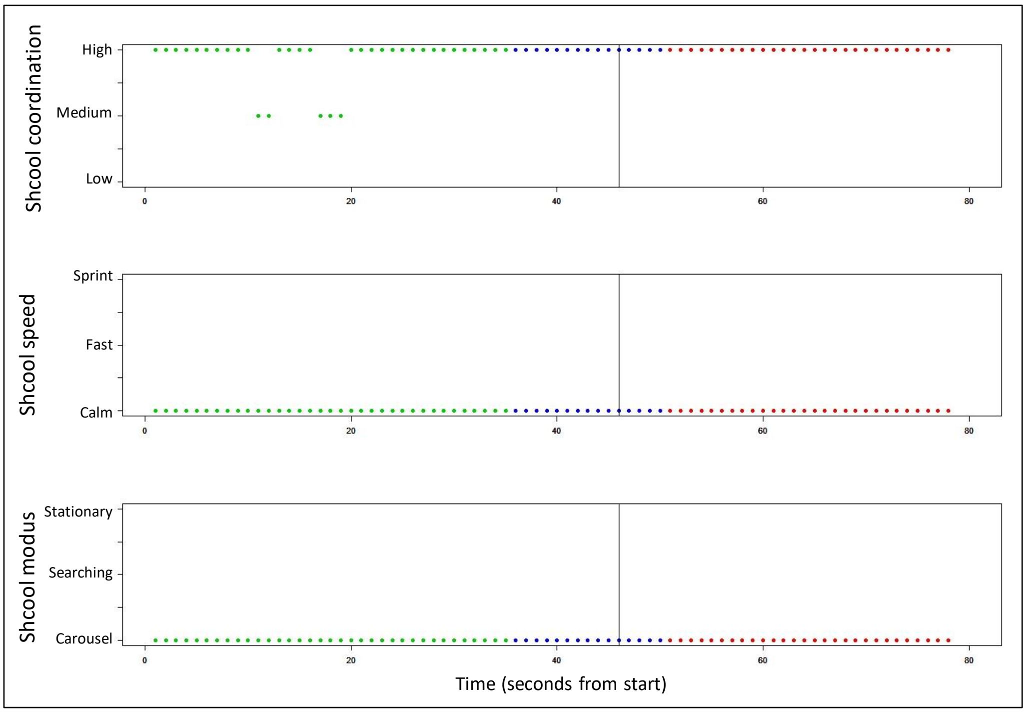

Figure C3. *Time series plots for the one block with control (sourse ship without seismic); block 5, run 4. Green points are the Pre period (before start), blue are during shooting, orange are between succeeding runs and red are the post exposure (after ended shooting). Black vertical line indicate CPA.*

7.4 Appendix D: Image sequences for manual tracking for swimming speed

Block 6 run 1 (dose escalation)

Experimental phase	Relative to pulse	Description	Start time	Start frame	End frame
Pre exposure		1. pulse - 30 min	09:46:38		
Pre exposure		1. pulse - 25 min	09:51:38		
Pre exposure		1. pulse - 20 min	09:56:38		
Pre exposure		1. pulse - 15 min	10:01:38		
Pre exposure		1. pulse - 10 min	10:06:38		
Pre exposure		1. pulse - 5 min	10:11:38		
Exposure	During pulse	1. pulse	10:16:33,00		

Exposure	Between pulses	REF - 1. pulse + 5sec	10:16:38		
Exposure	During pulse	2. pulse	10:16:43,00	45250	45300
Exposure	Between pulses	2. pulse + 5 sec	10:16:48,00	45375	45450
Exposure	During pulse	3. pulse	10:16:53,00	45500	45550
Exposure	Between pulses	3. pulse + 5 sec	10:16:58,00	45625	45700
Exposure	During pulse	1. pulse + 5 min	10:21:32,19	52493	52543
Exposure	Between pulses	1. pulse + 5 min + 5sec	10:21:37,18	52618	52693
Exposure	During pulse	1. pulse + 10 min	10:26:32,13	59988	60038
Exposure	Between pulses	1. pulse + 10 min + 5sec	10:26:37,13	60113	60188
Exposure	During pulse	1. pulse + 15 min	10:31:32,07	67482	67532
Exposure	Between pulses	1. pulse + 15 min + 5sec	10:31:37,07	67607	67682
Exposure	During pulse	1. pulse + 20 min	10:36:32,02	74977	75027
Exposure	Between pulses	1. pulse + 20 min + 5 sec	10:36:37,02	75102	75177
Exposure	During pulse	1. pulse + 25 min	10:41:31,21	82471	82521
Exposure	Between pulses	1. pulse + 25 min + 5 sec	10:41:36,21	82596	82671
Exposure	During pulse	1. pulse + 30 min	10:46:31,15	89965	90015
Exposure	Between pulses	1. pulse + 30 min + 5 sec	10:46:36,15	90090	90165
Exposure	During pulse	1. pulse + 35 min	10:51:31;08	97458	97508
Exposure	Between pulses	1. pulse + 35 min + 5 sec	10:51:36;08	97583	97658
Exposure	During pulse	1. pulse + 40 min	10:56:31,02	104952	105002
Exposure	Between pulses	1. pulse + 40 min + 5 sec	10:56:36,02	105077	105152
Exposure	During pulse	1. pulse + 45 min	11:01:30,21	112446	112496
Exposure	Between pulses	1. pulse + 45 min + 5sec	11:01:35,21	112571	112646
Exposure	During pulse	CPA 1	11:06:10,20	119445	119495
Exposure	Between pulses	CPA 1 + 5 sec	11:06:15,20	119570	119645

Exposure	During pulse	CPA 2	11:06:20,20	119695	119745
Exposure	Between pulses	CPA 2 + 5 sec	11:06:25,20	119820	119895
Exposure	During pulse	1. pulse + 50 min	11:06:30,20	119945	119995
Exposure	Between pulses	1. pulse + 50 min + 5sec	11:06:35,20	120070	120145
Exposure	During pulse	CPA 4	11:06:40,20	120195	120245
Exposure	Between pulses	CPA 4 + 5 sec	11:06:45,20	120320	120395
Exposure	During pulse	1. pulse + 55 min (6. siste puls)	11:11:31,10	127460	127510
Exposure	Between pulses	1. pulse + 55 min + 5sec	11:11:36,10	127585	127660
Post exposure		Last puls + 5 min	11:17:21,13	136213	136288
Post exposure		Last puls + 10 min	11:22:21,13	143713	143788
Post exposure		Last puls + 15 min	11:27:21,13	151213	151288
Post exposure		Last puls + 20 min	11:32:21,13	158713	158788

Block 7, run 1 (dose escalation)

Experimental phase	Relative to pulse	Description	Start time	Start frame	End frame
Pre exposure		1. pulse - 25 min	12:06:58,21	19015	19090
Pre exposure		1. pulse - 20 min	12:11:58,21	26515	26590
Pre exposure		1. pulse - 15 min	12:16:58,21	34015	34090
Pre exposure		1. pulse - 10 min	12:21:58,21	41515	41590
Pre exposure		1. pulse - 5 min	12:26:58,21	49015	49090
Exposure	During pulse	1. pulse - TIME REF	12:31:58,21	56515	56565
Exposure	Between pulses	1. pulse + 5sec	12:32:03,21	56640	56715
Exposure	During pulse	2. pulse	12:32:08,21	56765	56815
Exposure	Between pulses	2. pulse + 5 sec	12:32:13,21	56890	56965
Exposure	During pulse	3. pulse	12:32:18,21	57015	57065

Exposure	Between pulses	3. pulse + 5 sec	12:32:23,21	57140	57215
Exposure	During pulse	1. pulse + 5 min	12:36:58,16	64010	64060
Exposure	Between pulses	1. pulse + 5 min + 5sec	12:37:03,16	64135	64210
Exposure	During pulse	1. pulse + 10 min	12:41:58,11	71505	71555
Exposure	Between pulses	1. pulse + 10 min + 5sec	12:42:03,11	71630	71705
Exposure	During pulse	1. pulse + 15 min	12:46:58,06	79000	79050
Exposure	Between pulses	1. pulse + 15 min + 5sec	12:47:03,06	79125	79200
Exposure	During pulse	1. pulse + 20 min	12:51:58,01	86495	86545
Exposure	Between pulses	1. pulse + 20 min + 5 sec	12:52:03,01	86620	86695
Exposure	During pulse	1. pulse + 25 min	12:56:57,21	93990	94040
Exposure	Between pulses	1. pulse + 25 min + 5 sec	12:57:02,21	94115	94190
Exposure	During pulse	1. pulse + 30 min	13:01:57,16	101485	101535
Exposure	Between pulses	1. pulse + 30 min + 5 sec	13:02:02,16	101610	101685
Exposure	During pulse	1. pulse + 35 min	13:06:57,10	108979	109029
Exposure	Between pulses	1. pulse + 35 min + 5 sec	13:07:02,10	109104	109179
Exposure	During pulse	1. pulse + 40 min	13:11:57,04	116473	116523
Exposure	Between pulses	1. pulse + 40 min + 5 sec	13:12:02,04	116598	116673
Exposure	During pulse	1. pulse + 45 min	13:16:56,23	123967	124017
Exposure	Between pulses	1. pulse + 45 min + 5sec	13:17:01,23	124092	124167
Exposure	During pulse	CPA 1	13:21:56,18	131462	131512
Exposure	Between pulses	CPA 1 + 5 sec	13:22:01,18	131587	131662
Exposure	During pulse	CPA 2	13:22:06,18	131712	131762
Exposure	Between pulses	CPA 2 + 5 sec	13:22:11,18	131837	131912
Exposure	During pulse	CPA (1. pulse + 50 min)	13:22:16,18	131962	132012
Exposure	Between pulses	CPA (1. pulse + 50 min) + 5sec	13:22:21,18	132087	132162

Exposure	During pulse	CPA 4	13:22:26,18	132212	132262
Exposure	Between pulses	CPA 4 + 5 sec	13:22:31,18	132337	132412
Exposure	During pulse	1. pulse + 55 min (6. siste puls)	13:26:56,24	138968	139018
Exposure	Between pulses	1. pulse + 55 min + 5sec	13:27:01,24	139093	139168
Post exposure		Last puls + 5 min	13:35:57,12	152481	152556
Post exposure		Last puls + 10 min	13:40:57,12	159981	160056
Post exposure		Last puls + 15 min	13:45:57,12	167481	167556

Block 8, run 1 (seismic short)

Experimental phase	**Relative to pulse (or sequence length)**	**Description**	**Start time**	**Start frame**	**End frame**
Pre exposure	2 sec	1.pulse - 5min	13:44:14,10	164904	164954
Pre exposure	3 sec	1.pulse - 5min + 5sec	13:44:19,10	165029	165104
Pre exposure	2 sec	1.pulse - 2.5min	13:46:44,10	168654	168704
Pre exposure	3 sec	1.pulse - 2.5min + 5sec	13:46:49,10	168779	168854
Exposure	During pulse	1. pulse - TIME REF	13:49:14,10	172404	172454
Exposure	Between pulses	1. pulse + 5sec	13:49:19,10	172529	172604
Exposure	During pulse	2. pulse	13:49:24,10	172654	172704
Exposure	Between pulses	2. pulse + 5 sec	13:49:29,10	172779	172854
Exposure	During pulse	3. pulse	13:49:34,10	172904	172954
Exposure	Between pulses	3. pulse + 5 sec	13:49:39,10	173029	173104
Exposure	During pulse	Halfway 1.pulse	13:52:24,11	177155	177205
Exposure	Between pulses	Halfway 1.pulse + 5 sec	13:52:29,11	177280	177355
Exposure	During pulse	Halfway 2.pulse	13:52:34,11	177405	177455
Exposure	Between pulses	Halfway 2.pulse + 5 sec	13:52:39,11	177530	177605

Exposure	During pulse	Halfway 3.pulse	13:52:44,11	177655	177705
Exposure	Between pulses	Halfway 3.pulse + 5 sec	13:52:49,11	177780	177855
Exposure	During pulse	3rd last pulse	13:55:24,17	181661	181711
Exposure	Between pulses	3rd last pulse + 5sec	13:55:29,17	181786	181861
Exposure	During pulse	2nd last pulse	13:55:34,18	181912	181962
Exposure	Between pulses	2nd last pulse	13:55:39,18	182037	182112
Exposure	During pulse	Last pulse	13:55:44_18	182162	182212
Exposure	Between pulses	Last pulse + 5sec	13:55:49_18	182287	182362
Post exposure	2 sec	2.5min after last pulse	13:58:14,18	185912	185962
Post exposure	3 sec	2.5min after last pulse + 5sec	13:58:19,18	186037	186112
Post exposure	2 sec	5min after last pulse	14:00:44,18	189662	189712
Post exposure	3 sec	5min after last pulse + 5 sec	14:00:49,18	189787	189862

Retur: Havforskningsinstituttet, Postboks 1870 Nordnes, NO-5817 Bergen

HAVFORSKNINGSINSTITUTTET
Institute of Marine Research

Nordnesgaten 50 – Postboks 1870 Nordnes
NO-5817 Bergen
Tlf.: +47 55 23 85 00 – Faks: +47 55 23 85 31
E-post: post@imr.no

www.hi.no

HAVFORSKNINGSINSTITUTTET