Effects of seismic shooting on the lesser sandeel (*Ammodytes marinus* Raitt) – a field study with grab sampling and in *situ video* observations

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Acknowledgements

This thesis is submitted in partial fulfilment of the requirements for the degree of Candidatus scientarium in Fisheries Biology at the Department of Biology at the University of Bergen. The thesis has been linked up to a large project at the Institute of Marine Research in Bergen, and some of the results have already been published in Fisken og Havet and in ICES Journal of Marine Science.

I was early included in the project team working on the planning process for the experiment, and I was lucky to join the survey conducted early May 2002. My task during the field survey was to manage the grab sampling, control the video recordings and handle the caged fish when brought onboard. Later on I have had the responsibility for the video analyses by developing analysis, methods and performing the analyses. In addition, I have analysed the sand samples, grab sampling and been a co-writer on the earlier mentioned report and paper. Some of the figures, data and results are therefore reproduced in this thesis.

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Abstract

Seismic shooting with airguns has been conducted continuously in the North Sea for about 40 years. In the past two decades there has been conflicts of interest between Norwegian industrial trawlers fishing for sandeel and seismic exploration activities in the North Sea. The fishermen have claimed that seismic shooting scares fish to flee to the bottom where the burry deep in the sediments and die. A common experience is that catchability is reduced for a period after seismic shooting. In May 2002, a field experiment on the effect of seismic shooting on the lesser sandeel (*Ammodytes marinus*) was conducted in the southeastern part of the North Sea. Buried sandeel were trapped in customized, bottomless, steel frame cages deployed during night on aggregations of sandeel located by a van Veen grab. Three cages were deployed in an experimental area and exposed to sounds from a standard three-dimensional (3D) seismic survey for about 2.5 days. The behaviour of the fish caged fish were monitored and recorded with cameras mounted in one of the cage and on a remotely operated vehicle (ROV). A control area with two cages was established about 35 km southeast of the experimental area. Within the shooting area, a predetermined set of localities was repeatedly sampled by grab (van Veen) prior to and after seismic shooting.

The seismic shooting had no immediate lethal effect on the sandeel. No dead sandeel was found in the grab samples and the total mortality in the cages was on average about 35 % both in the experimental and control groups. This mortality was probably a result of injuries due to handling and confinement. There was no significant difference in the catches of sandeel in the grab samples before and after shooting. Behaviour analyses from the video recordings showed that the caged fish did not flee to the bottom exposed to seismic shooting. They reacted to the shooting by performing C-starts and increased tailbeat frequency. Almost all C-starts were recorded during seismic shooting, none was recorded before and only one was recorded after. The tailbeat frequency was significantly higher both during and after than prior to seismic shooting and it did not return to preshooting levels during the 3-day period after the shooting ended. Some of the results differ from other results found in comparable studies. The sandeel lacks swimbladder and are thought to have poor hearing abilities and this is suggested to be an explanation for these differences.

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1 Introduction

Since the beginning of the oil activity in the North Sea in the early 1960s, there have been conflicts of interest between the fisheries and the oil industry. A constant source of dispute is the seismic exploration activities, which are essential for the localisation of offshore oil and gas reservoirs. Seismic exploration activities and later, oil and gas production, have taken place and still take place in areas with significant fishing activities. In the past two decades there has been disagreement between Norwegian industrial trawlers fishing for sandeel and the oil industry in the North Sea. The fishermen have long claimed that seismic shooting scares fish to flee to the bottom where they burry deep in the sediments and die. A common experience amongst the fishermen is that catchability is reduced for a period of time after seismic shooting. Scientific studies to confirm these statements have not yet been conducted.

The sandeel fishery has become an important income for the Norwegian industrial trawlers and many fishermen are now dependent on this resource. The commercial fishery for sandeel began in the early 1950s and remained at relatively modest levels until the mid 1960s, when annual catches were below 200.000 tonnes. Towards the mid 1970s catches rose dramatically in connection with declining catches of other pelagic fish, especially herring (*Clupea harengus*). Since 1975, the sandeel catches have generally fluctuated between 600.000 and 900.000 tonnes, with some years exceeding one million tonnes (Anon., 2003).

The most important fishing grounds, indicating the largest concentrations of sandeel, are found in the central parts of the North Sea and from the Dogger Bank northwards along the coast of England and Scotland. The Norwegian industrial trawler fishery has largely taken place in central regions of the North Sea, more specifically the Eastern Bank, Klondyke and Inner and Outer Shoals, and in this fishery, the lesser sandeel account for more than 95% of the catch. In the central region of the North Sea there has been and still is a high seismic activity. The area between N 55°-62° has been explored by seismic surveys since the beginning of the oil activity, and in 1974 about 40.000 line km were surveyed. In the following years the exploration effort increased and in 1994 a top was reached when almost 550.000 line km were shot. Since then, the effort in the central region of the North Sea has decreased, but the area is still a common exploration area with over 110.000 line km being shot in 2003 (B. Randeberg, The Norwegian Petroleum Directorate, *pers. comm.*, 2004).

The name “sandeel” (also named “sand lance”) is a collective term of about twelve species (Nelson, 1984) in the family Ammodytidae. Five species are registered in the North Sea, and these are the lesser sandeel (*Ammodytes marinus*), smooth sandeel (*Gymnammodytes semisquamatus*), small sandeel *(Ammodytes tobianus*), greater sandeel (*Hyperoplus lanceolatus*) and Corbin`s sandeel (*Hyperoplus immaculatus*). Several of these species play important roles in the marine food chain. Nutritional ecology studies have shown that these species are important for most large fish species in the coastal ecosystem, i.e. for cod (*Gadus morhua*), saithe (*Pollachius* virens), haddock (*Melanogrammus aeglefinus*) and flatfish species, particularly plaice (*Pleuronectes platessa*) (Høines *et al.*, 1995). In the North Sea, the lesser sandeel is also the dominant prey of seabirds such as cormorants and auks (Baily *et al.*, 1991).

The individual sandeel species display some differences in their biology with regard to behaviour, growth patterns, spawning season and age at first spawning. The following paragraphs describe the distribution, biology and behaviour of the lesser sandeel, since this species is the most important both in terms of abundance and importance for the commercial industrial fishery.

The lesser sandeel is the most common fish species in the North Sea, and is widely distributed throughout the whole of the North Sea region, except the deeper parts of the Norwegian trench. Lesser sandeel occurs in large numbers all along the coast up to the Kola Peninsula. We also find this species near the Faeroes, Iceland and Greenland. In the Baltic it is found as far east as Bornholm. The sandeel inhabits sandy bottoms, ranging from fine sand to coarse shell sand, which share the characteristic of good oxygen conditions in the substrate. Sandeel density can be extremely high in the most suitable areas. In January 1998, on the Inner Shoal in the northern North Sea, experiments were carried out collecting buried sandeels using a van Veen grab, with mean catch rates of 61 individuals per square meter (Høines and Bergstad, 2001). When the same spot was revisited in January 1999 no sandeels were caught, indicating the wide variations in density that can occur.

In Denmark, the sandeel is also known as the “sand badger”, a name that better reflects its biological peculiarities. This is because the sandeel is specialized on living on sandy bottoms and it spends most of the time partially buried in the sediment. It lacks a swimbladder, which means that it needs to constantly move to avoid sinking when it is in the water column. The sandeel has a torpedo-shaped body with a protruding tip on the lower jaw making it particularly suited for burying into the sand. During the winter the sandeel is buried in the sand in a state of hibernation, only interrupted by the spawning period in December/January. In the summer, when it is otherwise active, it spends both the hours of darkness and dark cloudiness in this manner (Popp Madsen, 1994).

The sandeel feeds on different kinds of zooplankton, which are sucked in when the mouth is opened. The upper jaw parts are connected in such way that the whole of the mouth can be shot forward to form a sort of tube, creating a vacuum in the buccal cavity (Macer, 1966; Popp Madsen, 1994). The feeding period is mainly from the early morning and throughout the day, i.e. the fish emerge from the sand relatively synchronously in the morning and feed throughout the day, returning to the sand when it is satiated or when the availability of food decreases. This means that the sandeel returns to the sand in a relatively unsynchronised pattern throughout the day (Winslade, 1974; Popp Madsen, 1994).

Fish, like other animals, are constantly influenced by physical and chemical stimuli like light, sound, pressure, odour and taste. These stimuli are registered by the sense organs, and can initiate behaviour patterns such as mating, foraging or predator avoidance. Sound in water appears different than in air, because water has greater density, lower elasticity and higher sound propagation velocity than air. This means that sound propagates rapidly and effectively through water, and can provide essential information. Underwater sound is generally important to fish. A wide range of species emits sounds (Myrberg, 1981), and many species are shown to be acutely sensitive to underwater sounds (Hawkins, 1993). If the fish shall detect sound, the level of the stimulus must exceed the ambient noise level (Engås *et al.*, 1996), which is about 80-90 dB re 1 μPa Hz^{-1} in open sea (Wenz, 1962).

Underwater sound and the ability to hear in a marine environment have in the recent years obtained much attention. One question is how fish react to human generated sound, and there has become a growing concern that these sounds may have a substantial impact on fish and other marine organisms (e.g., Dalen *et al.*, 1996; Anon., 2000; Popper, 2003). Seismic air guns, used in marine petroleum explorations produce a repetitive short, sharp, low-frequency sound (10-200 Hz, Malme, 1986; Gausland, 1998), which is within the auditory range of all investigated marine fish species (Hawkins, 1993). The auditory range for sandeel or any close relatives has not been established, but some work has been done on other species lacking swimbladder. The auditory sensitivity of dab (*Limanda limanda*) is for instance best in the frequency band 110-160 Hz and the hearing threshold has been determined to be about 92 dB re 1 μPa (Chapman and Sand, 1974; Hawkins, 1993). A typical peak source level for a large air gun array is between 250 and 255 dB re 1 μPa (Greene, 1985; Gausland, 2000), a level that is far above the hearing threshold of all tested marine fish species (Hawkins, 1993).

No research has been done on how sandeel reacts to seismic activity, but some seismic/sound studies have been done on other species. Herring showed a marked startle response when subjected to sound stimuli from a vibrating source (Blaxter *et al.*, 1981). Dalen and Knutsen (1987) have suggested that cod swim towards the bottom when exposed to seismic signals and Pearson *et al.* (1992) observed that rockfish (*Sebastes* spp.) aggregated near the bottom during airgun discharges. In the same study, airgun discharges were also reported to elicit startle and alarm responses in the fish. In trials off the coast of California hook and line catch rates for rockfish were reduced by 50 % under the influence of a single air gun (Skalski *et al.,* 1992). When investigating catch data obtained from commercial vessels operating on fishing grounds where seismic explorations were being conducted, Løkkeborg and Soldal (1993) found a 55-85 % reduction in longline catches of cod and a reduction of 80-85 % in the by-catch of cod in shrimp trawling, Engås *et al.* (1996) found that seismic shooting severely affected distribution, local abundance and catch rates of cod and haddock within a distance of 18 nautical miles (~33 km). Wardle *et al.* (2001) observed involuntary reactions in form of a Mauthner cell reflex in saithe and pollack (*Pollachius pollachius*) when firing a seismic airgun. The fish turned and fled from the explosion when the explosion source was visible, but when the source was not visible the fish continued swimming without changing direction.

This thesis is a result of a large field experiment that was carried out in May 2002 to study the influence of seismic shooting on lesser sandeel on a shallow North Sea fishing ground. The study was based on acoustic surveying during daytime, grab sampling at night and *in situ* video observations of the behaviour of fish trapped in cages, before, during and after a standard three-dimensional (3D) seismic survey. This thesis concentrates on the grab sampling and the analyses of the *in situ* video observations of the caged sandeel.

Seismic shooting may influence the behaviour of the sandeel in different ways. The fish can either flee or stay in the area. If they stay, they can burry in the sand, or swim in the water column. If they keep swimming, they can move vertically, i.e. swim to the upper, lower or middle part of the water column. They may also increase their activity level, change swimming direction and perform involuntary reactions like Mauthner cell reflexes.

The experiment was designed to answer these questions. Grab sampling at night can reveal if the sandeel stays in the area or flees. Grab sampling can also give information on whether the fish burry in the sand or stay in the water column during the day. The *in situ* video observations will give information on their vertical location in the water column, and any other possible changes in the behaviour.

2 Material and methods

2.1 Design and set-up of the experiment

In order to study behaviour and survival of sandeel exposed to seismic shooting, six cages were especially designed for the purpose of trapping free-living sandeel when they were buried in the sand, such that they could be observed before, during and after a standard seismic survey. In addition, the cages were designed for bringing both dead and live sandeel to the surface for further examination at the end of the experiment. Three of the cages should be deployed in an experimental area with seismic activity and the other 3 in a control area at least 33 km away from the experimental area (Engås *et al.*, 1996). For behavioural observations, one cage in each area was equipped with a fixed camera mounted inside of the cage, and an ROV (Remotely Operated Vehicle) operating in both areas was also used for inspection and observations of the caged fish.

Grab sampling at night was used to locate suitable areas for deployment of the cages, and an area within the shooting region was sampled before and after the shooting to detect possible changes in the distribution, abundance and mortality of sandeel.

2.2 Period and study site

The field experiment was carried out in the period 1-20 May 2002 on board the research vessel R/V “Håkon Mosby”. Based on experiences from earlier investigations (Høines and Bergstad, 2001) and information from commercial fishing vessels, the investigation area was chosen in the southern North Sea in an area called “The Diana ground”, centred at N57°12.5` E05°19.1`(Figure 2.1). An area about 35 km to the southeast located at N56°55.4` E05°41.0` was selected to be the control area.

A chartered seismic vessel conducted seismic shooting from 13 May at 12:30 until 15 May at 20:13. All time units are given in UTC + 2 hours (UTC = Coordinated Universal Time). Details and information about seismic equipment and shooting procedure are given in chapter 2.6.

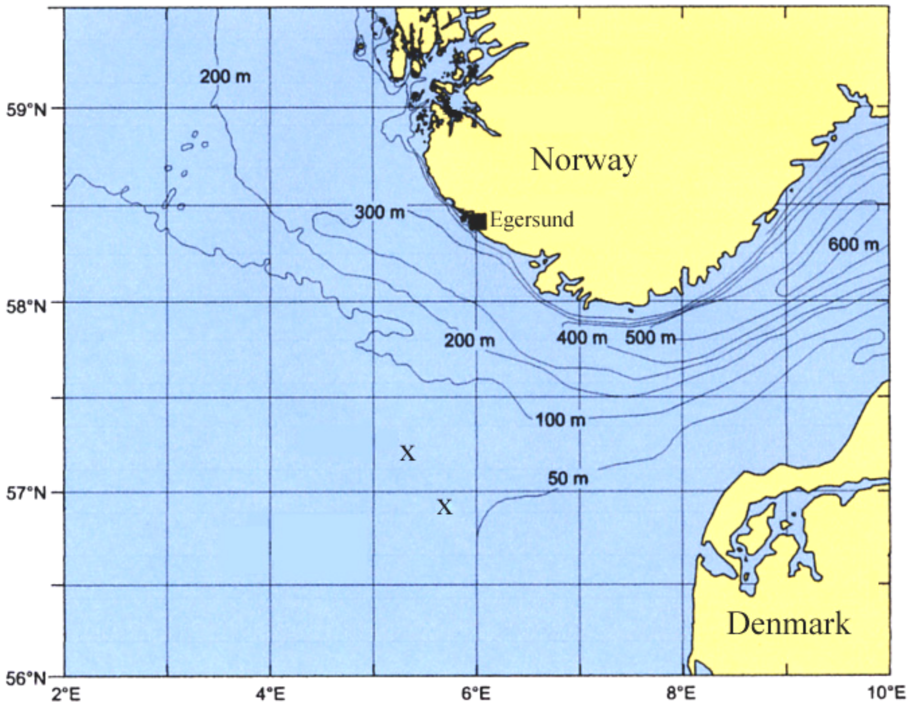

Figure 2.1. Location of the experimental and control area (upper and lower cross, X, respectively) southwest of Egersund on the Diana ground.

The depths in the experimental and control area were 53 and 49 m respectively, and the sediment consisted of fine (0.25 mm) sand in both areas. The temperature at the bottom was 7°C during the whole period, and the salinity was 35.1 psu. Depth, temperature and salinity were read from an environmental sensing system (CTD) placed on the ROV.

2.3 The cages

2.3.1 Technical description and function of the cages

The cages consisted of a framework mounted on a base frame. Inside of the framework, a sandeel net (the same as used by the sandeel fleet) was attached. The general design is illustrated in Figure 2.2. The dimensions of the cages were calculated according to physical models (NORWECOM) (Svendsen *et al.*, 1996), showing that the cages had to withstand an hourly average current of 14 cm^{-1} at 50-70 m depth. The framework was constructed of 50 mm steel pipes with length, width and height 2.0 x 2.0 x 1.8 m, respectively. The base frame was made of 12 mm welded steel plate measuring 2.5 x 2.0 x 0.3 m.

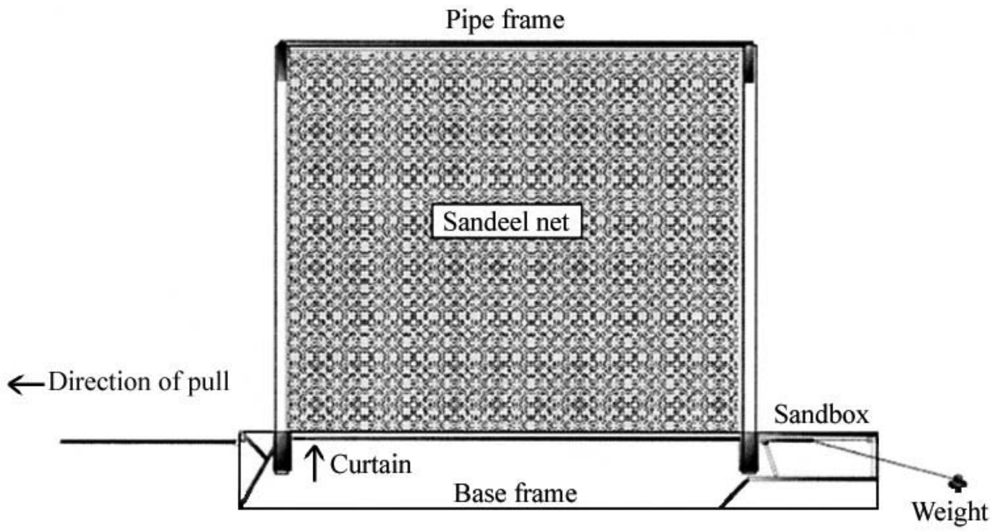

Figure. 2.2. General design of the cage. A pipe frame was placed on a base frame. The function of the sandbox and curtain is explained in text. Illustration: Redrawn from Hassel *et al.*, 2003, with permission.

The cages were designed to catch sandeel when lowered to the seabed. Accordingly, the cages were made without bottom in such a way that the sandeel, which is buried in the sand during night, were trapped when the cages were placed on the seabed. To prevent fish from escaping under the framework, the steel edge on the base frame had to sink into the sand. The cages were thus constructed with sufficient weight to sink 10 cm into the sediment, and to make the cage penetrate the substrate, the steel edge on the frame was sharpened, ranging gradually from 12 to 3 mm. When the sandeels were trapped in the cage, observations could be done by a fixed camera mounted inside one of the cages and by cameras on the ROV. The cages were equipped with an inspection window (1.0 x 0.5 m) made of plastic and sewn into the sandeel net through which the ROV could observe (Figure 2.3).

Figure. 2.3. Left: The inspection window sewn into the sandeel net. The picture also shows the pipe frame and the sandbox. Right: A picture taken from the ROV's camera when approaching the inspection window.

After the observation period, the cages and all fish, both dead and alive, had to be brought to the surface and on board the vessel. To collect dead fish lying at the bottom and fish buried in the sand (dead and alive), the cages were equipped with a scrape-function and a ventilated box, named “sandbox”. The scrape-function was a 45° cutting edge towards the bottom, and when the cage was pulled horizontally the top layer (5-10 cm) with sand and fish was transported and guided into the sandbox. The sandbox was ventilated with drilled holes (Ø 0.5 cm), thus separating the sand from the sandeels. To avoid that the content of the box slipped out, the entrance of the sandbox was equipped with a hinged one-way door that only opened inwards.

To get swimming fish safely on board, a technical solution called “curtain” was developed. The curtain was made from sandeel net and when unfolded it made a floor in the cage. It was stored inside the frame at the opposite side of the sandbox, and was pulled out by three wires, going from the curtain through holes in the sandbox and then attached to a 2.0 m long steel bar, which was connected to a 20 kg chain. When pulling the cage horizontally, i.e. scraping the top-layer of the sediment, the steel bar and chain remained still in their position on the seabed and thus closed the curtain.

2.3.2 Deployment

To ensure that sandeel was present in the sand before deploying the cages, the bottom was examined by grab (van Veen 0.2 m^2) and ROV. After finding a spot with buried sandeels, the deployment of the cages began. First a buoy line with 290 kg load (railway carriage wheel) was deployed and then 200 m of rope going from the load to the front of the cage frame (opposite to the sandbox). The use of the load made it possible to pull the cage horizontally along the seabed during retrieval (Figure 2.4). The cage was lowered to bottom using ropes attached to the corners on top of the cage. It was important that the curtain remained unfolded during deployment, and it was therefore secured with magnesium screws, which dissolves after 24 hours immersion in seawater. The steel bar with the attached chain was secured with two ropes, one at each end of the bar. The cages were placed within a range of about 300 m.

Two of the cages in the experimental area were deployed in the evening of 4 May and the third one (cage 3a) at midnight 6 May. Because of technical problems, cage 3a had to be retrieved 12 May, but was redeployed the same day (cage 3b). Although it was placed in the experimental area cage 3a was used as a control, because it was retrieved before the seismic shooting started.

In the control area, cage 4 and 5 were deployed 12 May. The sandeel concentrations in the control area were too low to allow successful catching of fish by lowering the cages to the bottom. Instead fish were caught by trawl and immediately transferred to a bucket attached inside the cages. The bucket was covered with sandeel net that was secured by means of a magnesium bar, which dissolves within a few hours after exposure to seawater, releasing the fish into the cage. Positions and dates of deployment and retrieval of the cages are given in Table 2.1.

Table 2.1. Positions and dates of deployment and retrieval of the cages in the seismic and control area.

Cage no.	Cage	Latitude	Longitude	Date deployed	Time	Date retrieved	Time
1	Seismic	57.2097	5.3190	04.05.2002	23:42	19.05.2002	14:00
2	Seismic	57.2095	5.3160	04.05.2002	22:38	19.05.2002	11:20
3b	Seismic	57.2087	5.3200	12.05.2002	00:05	19.05.2002	09:05
3a	Control*	57.2092	5.3210	06.05.2002	23:59	12.05.2002	10:41
4	Control	56.9233	5.6833	12.05.2002	22:31	18.05.2002	15:05
5	Control	56.9220	5.6815	12.05.2002	23:13	18.05.2002	14:20

*Cage was placed in the seismic area, but was retrieved before shooting.

2.3.3 Retrieval

By using the mechanical arm on the ROV to cut the security ropes on the steel bar, the dragging weight, i.e. the steel bar and chain, was left on the bottom, and the pulling of the cage could begin. The cage was pulled horizontally at least 3 m to unfold the curtain. Then the load and the cage were hauled to the surface and taken onboard.

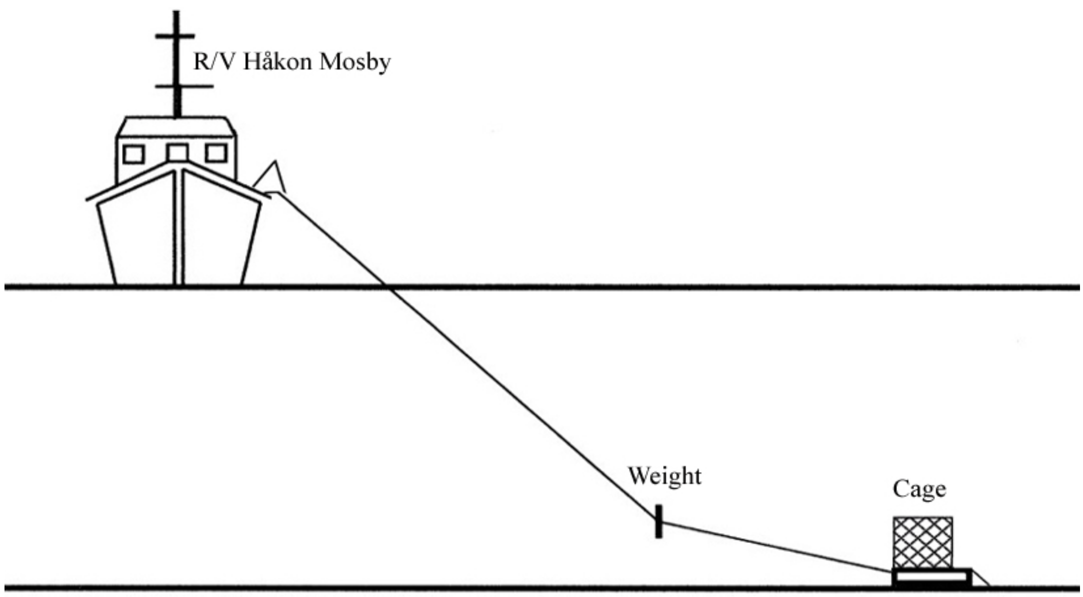

Figure. 2.4. Retrieval of the cage. The cage was pulled to the left to fill the sandbox and close the curtain before lifting the cage to the surface.

2.3.4 Definition of dead and alive sandeel

When a cage was retrieved and brought onboard, all sandeels were quickly transferred to a 50 litres bucket with fresh seawater. The sandeels were categorized as dead, when they had signs of decay and/or did not respond (moved) when they were held in the hand for 30 seconds. All fish showing any kind of movement were categorized as living. The number of fish in each cage were counted and measured (total length, nearest 0.5 cm below).

2.4 Grab sampling

A van Veen grab (0.2 m^2) was used to locate suitable areas for the experiment. After placing the cages, a confined area around the cages (Figure 2.5) was sampled before and after the shooting, in order to detect possible changes in behaviour or mortality of the sandeel as a result of the seismic activity. The sampling area was confined to avoid too close passage of the ropes and buoys connected to the cages. Sampling was only carried out when the weather was quite calm with no gale or storm. Sometimes the grab had closed before reaching the bottom because of uneven running of the winch or motion of the ship. Incomplete grabs are not included in the analyses. A total of 19 grab shots were taken before the seismic shooting began, and 59 grab shots were taken after the shooting. All shots were taken during the night, defined as the time between the end and the beginning of civil twilight. Civil twilight is defined to begin in the morning and end in the evening, when the centre of the sun is geometrically 6° below the horizon. According to this definition it was night in the period from 22:32-04:40 at 10 May 2002[1], when the field experiment was half way through.

For each successful grab sampling, a sediment sample was taken and the number of sandeels was recorded. In order to determine the particle size of the sediment, the sand samples were dried for 24 h at 100°C, and then sieved through a standard Retsch series of sieves ranging from 2000 to 63 µm mesh with the aid of a mechanical shaker.

[1] Data calculated from U.S. Naval Observatory (http://aa.usno.navy.mil/data/docs/RS_OneDay.html).

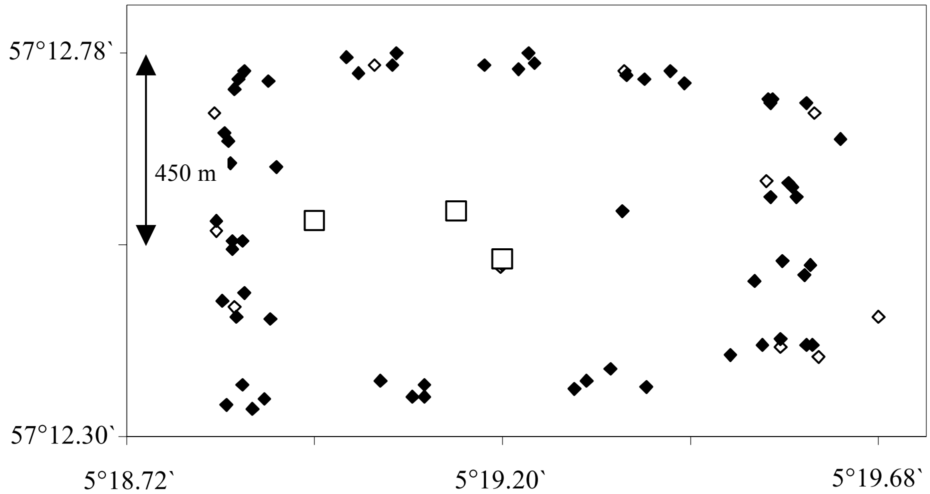

Figure 2.5. Grab shots taken during night in the area around the cages. Open squares, ◇, are grab shots taken before shooting and solid squares, ◆, are grab shots taken after shooting. The larger open squares, □, indicate the positions of the cages. Seven grab shots taken before shooting are not shown because of missing exact positions, but according to the log they were taken within the confined area. In addition, 2 of the grab shots taken before shooting are hard to see, because they were very close to the cage farthest south.

2.5 Video equipment and analysis methods

Observations on fish behaviour were obtained by using the camera (Panasonic high-resolution digital 3-ccd) on the ROV “Aglantha” (Figure 2.6 right), and a SIT (Silicone Intensified Tube) camera (Osprey 1323-124) mounted inside cage 3b. This camera was mounted in the upper corner to cover most of the bottom and 2/3 the distance up to the walls (Figure 2.6 left). The camera was connected by cable to a video link placed inside a plastic container floating at the surface. The container was supplied with batteries, and the video signals were transmitted to a monitor (Sony PVM-1442QM) and a VHS recorder (Panasonic NV-J35) on board RV “Håkon Mosby”. The container was also equipped with a VHS 24 h timelapse recorder (Vicon VCR424), allowing long play recordings of the fish when the boat was out of range of the video link.

Figure 2.6. Left: The SIT camera mounted in cage 3b. Right: ROV “Aglantha” on board R/V “Håkon Mosby”.

In the original set-up design for the experiment, there was planned to be three cages in the experimental area and three in the control area. In both areas, one of the cages should be equipped with a fixed mounted SIT camera connected to a container with a video link. The container could be equipped with a VHS 24 h timelapse recorder allowing recordings from the experimental area when the vessel was in the control area and vice versa. Due to technical problems, the camera cage in the control area was never deployed and the control area thus had only two cages. The recordings from this area could only be done by the ROV. The recordings from the control area were too few and of too low quality to allow comparison with the recordings from the experimental area, and the analyses therefore had to be based on comparisons of the recordings from the cages in the experimental area before, during and after the seismic shooting.

The ROV was able to make observations and monitor the fish at different angles. The analyses were, however, restricted to recordings with fixed conditions, i.e. the same positioning of the ROV, same use of camera zooming and no use of artificial light. The recordings from ROV “Aglantha”, named “Aglantha videos”, were divided into 10 minutes time blocks, where the picture was stable and the quality satisfactory from block to block. The time blocks are given in details in Appendix chapter 6.1.

All analyses were made at IMRs video lab on a Panasonic monitor (BT-S1460Y) and a Sony video player (SVO-1520P). The recordings were made on EMTEC EQ 260 videocassettes.

2.5.1 Behaviour of sandeel

The video observations were used to detect possible changes in the behaviour of sandeel when exposed to seismic shooting. The following aspects of the behaviour were quantified:

1) Vertical location of the sandeels in the cage
2) C-start reactions
3) Tailbeat frequency (tailbeats per second, b/s)

Category 1 was registered from the recordings done by the SIT camera inside cage 3, named “Video link videos” and “Timelapse videos”. Category 2 and 3 were recorded from the time blocks from the “Aglantha videos”.

Based on the fishermen’s statement that sandeel flee to the bottom and burry in the sediment during a seismic survey, the vertical location of the sandeels and the number of fish seen entering and leaving the sand were registered. At each whole minute the picture was frozen and the number of fish in the image was counted. The camera covered most of the bottom and 2/3 the distance up to the walls, but not the upper part and the roof of the cage. Knowing the total number of fish in the cage (the total number of fish were counted after retrieval) and under the assumption (see Discussion) that no fish were hiding in the sand and the not visible fish thus stayed in the upper part of the cage, it could be investigated if the fish changed their position in the cages during the experiment. The observed fish was defined to be in the lower part and the not seen in the upper part.

A behaviour pattern often observed in relation to seismic/sound is reactions involving jerking movements and bending of the body often followed by rapid swimming (Blaxter *et al.,* 1981; Pearson *et al.*, 1992; McCauley *et al.*, 2000; Wardle *et al.*, 2001). This behaviour is often called C-start, C-turn or startle response and is usually mediated by the Mauthner cells (Eaton *et al.,* 1977, 1981), and involves an involuntary contraction of the lateral muscles along one side. *In situ* observations during the survey revealed a similar reaction containing sudden jerk motions and bending of the body. This behaviour was defined as C-start and was counted from the time blocks before, during and after the seismic shooting. A general description of the reaction is illustrated in Results chapter 3.3.2, Figure 3.3.

Recording the frequency of tailbeats enabled quantification of the level of activity. Distance swum per time unit (i.e. swimming speed) was difficult to record as the fish swam in different directions and seldom in a straight angel to the camera. The distance between the camera and the fish was also unknown. When recording tailbeat frequency, the number of tailbeats of a randomly chosen fish was counted until the fish went out of visual range. No fish remained visual for more than 1 minute and a new fish was selected and counted 1 minute after the start of the previous count. In this way, the tailbeat frequency of 10 fish was measured from each time block. To select fish randomly, the monitor image was divided into six squares. A dice was thrown, and the fish in the square with number corresponding to the side of the dice was selected. Occasionally, when there were more than one fish in a square the most visible one was observed. A tailbeat was defined and counted as one beat when the caudal fin had moved from one side to the other one.

2.6 Seismic equipment

The seismic equipment and operation were typical for 3 D investigations on the Norwegian shelf and had equivalent acoustic performance and characteristics. Cooperation with the Norwegian seismic operator PGS AS was established and the seismic vessel M/V "Falcon Explorer" was hired.

The operative parameters of the airgun set-up are presented in Table 2.2. The airgun array was configured with 11 single airguns and 10 airgun clusters each of 2 guns. All together there were 31 airguns of which 3 guns were inactive (spare) (Figure 2.7). The practical execution of the shooting assignment was performed according to normal procedures, i.e. a shot was fired every 10 seconds, or every 25 meters. The survey was performed with soft-start procedure, which included a ~20 minutes period of ramping up air pressure before shooting the tracklines. This is done to allow possible marine mammals to move out of the area before the arrays are fired at full rate.

Table 2.2. Type and magnitudes of main parameters of the airgun set-up. Data from Schoolmeesters, 2002, with permission.

Parameters	Type/Magnitude
Array (source)	3090__60_2000_100
Airgun type	Bolt 1900 LLXT
Total source volume	50.6 l (3090 cu.in.)
Operation pressure	140 kg/cm^2
Depth of source	6.0 m
Distance between sub-arrays	12.5 m
Extension of array (lenght x width)	15 x 25 m

The ordinary expressed far field pressure signature of the airgun set-up is shown in Figure 2.8. Note that the near/far field transition range was approximately 9000 m from the array source. This means that the actual distances between the array and the fish in the experimental area were in the near field of the air-gun array. The sound pressure level (SPL) of the primary pulse amplitude of the far field (> 9000 m) was: SPL_{ff} = 256.9 dB re 1 μPa re 1 m. The more relevant near field pressure amplitude is displayed in Figure 2.9, and the SPL of the primary pulse of the near field (< 9000 m) was SPL_{nf} = 256.1 dB re 1 μPa re 1 m. Figure 2.10 shows the amplitude-frequency spectrum of the near field pressure signature (Figure 2.9) and illustrates how the sound energy was distributed by frequency.

Note that the figures (2.8 – 2.10) are from simulations based on mathematical models, which are found to be representative for real measured figures as evaluated by the seismic operators and the oil companies using controlled measurements.

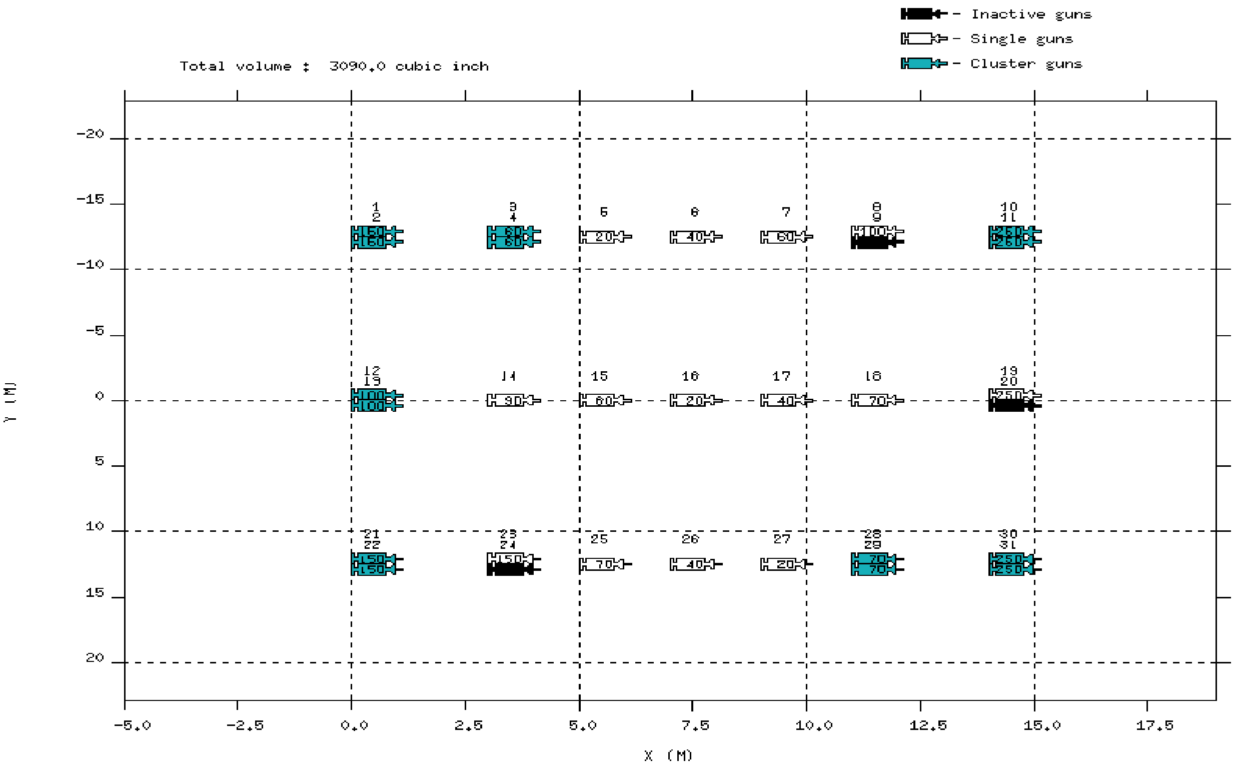

Figure 2.7. Configuration of the airgun array with single airguns, airgun clusters, active and inactive airguns. Inside each airgun symbol the volume of each gun is given in cubic inches. Illustration: From Schoolmeesters, 2002, with permission.

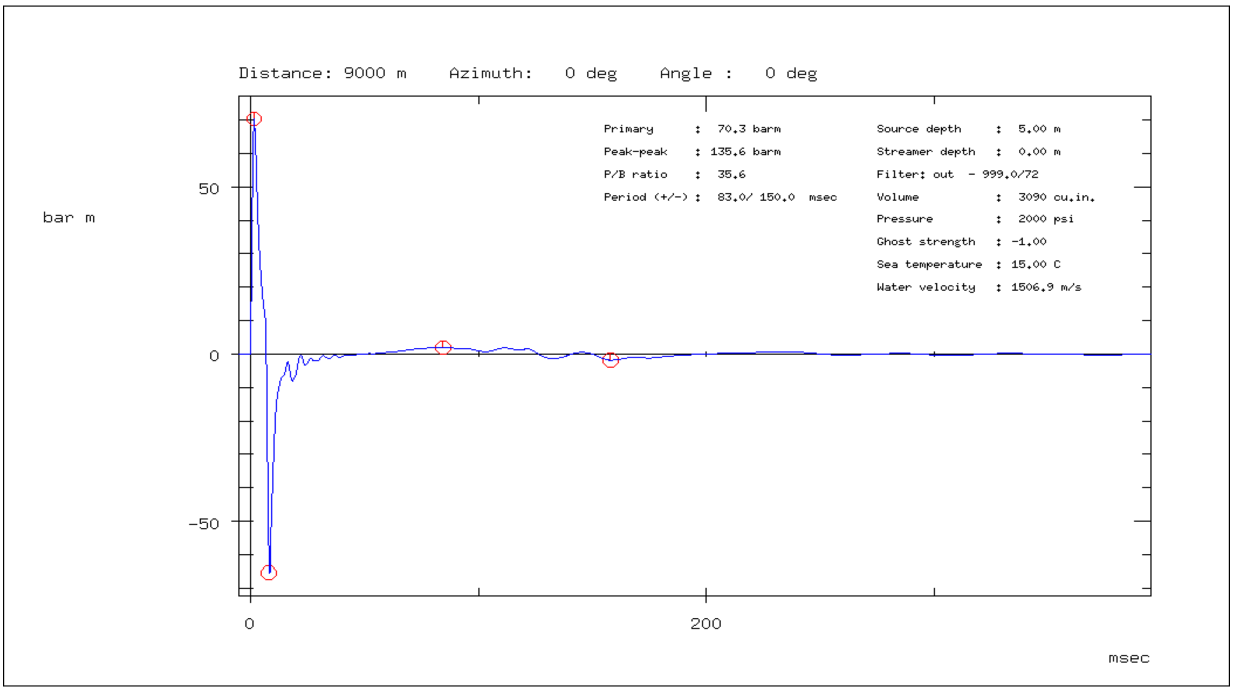

Figure 2.8. Far field pressure signature from the airgun array in bar-m (pressure in bar referred to 1 m from the source centre) versus time [ms]. Illustration: From Schoolmeesters, 2002, with permission.

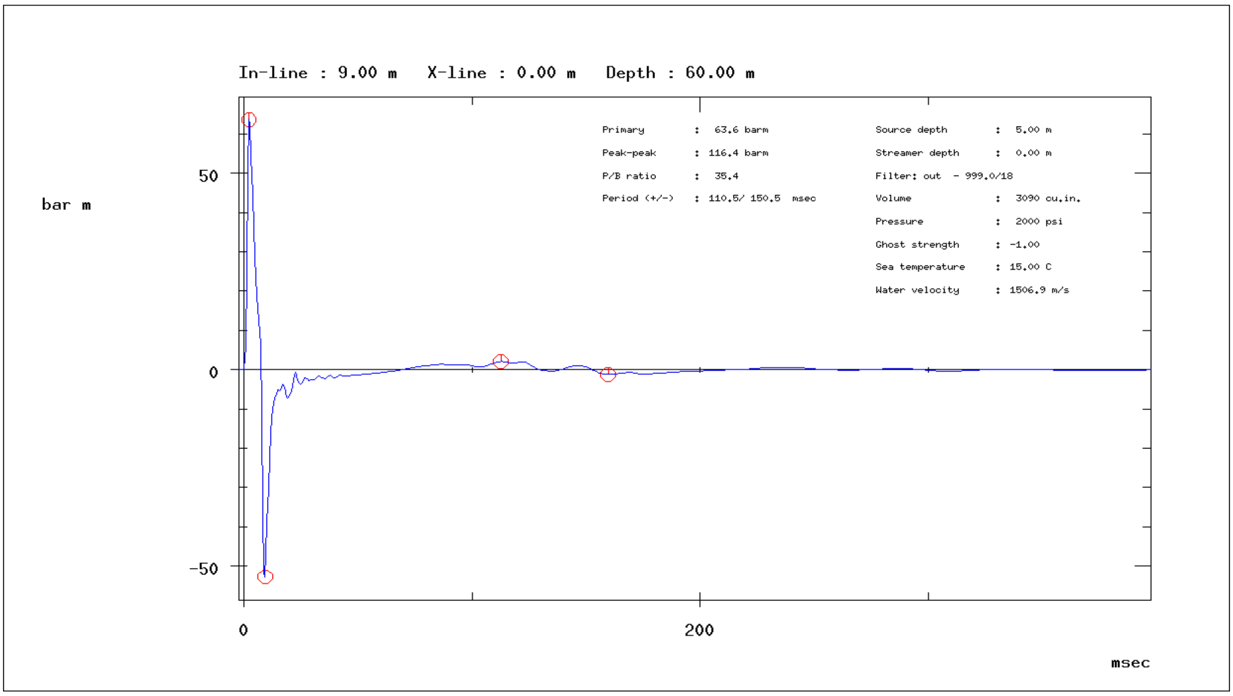

Figure 2.9. Near field pressure signature from the airgun array in bar-m (pressure in bar referred to 1 m from the source centre) versus time [ms]. Measurement position is x = 9.0 m, y = 0.0 m and depth, z = 60.0 m, which means vertically underneath the centre of gravity of the array. Illustration: From Schoolmeesters, 2002, with permission.

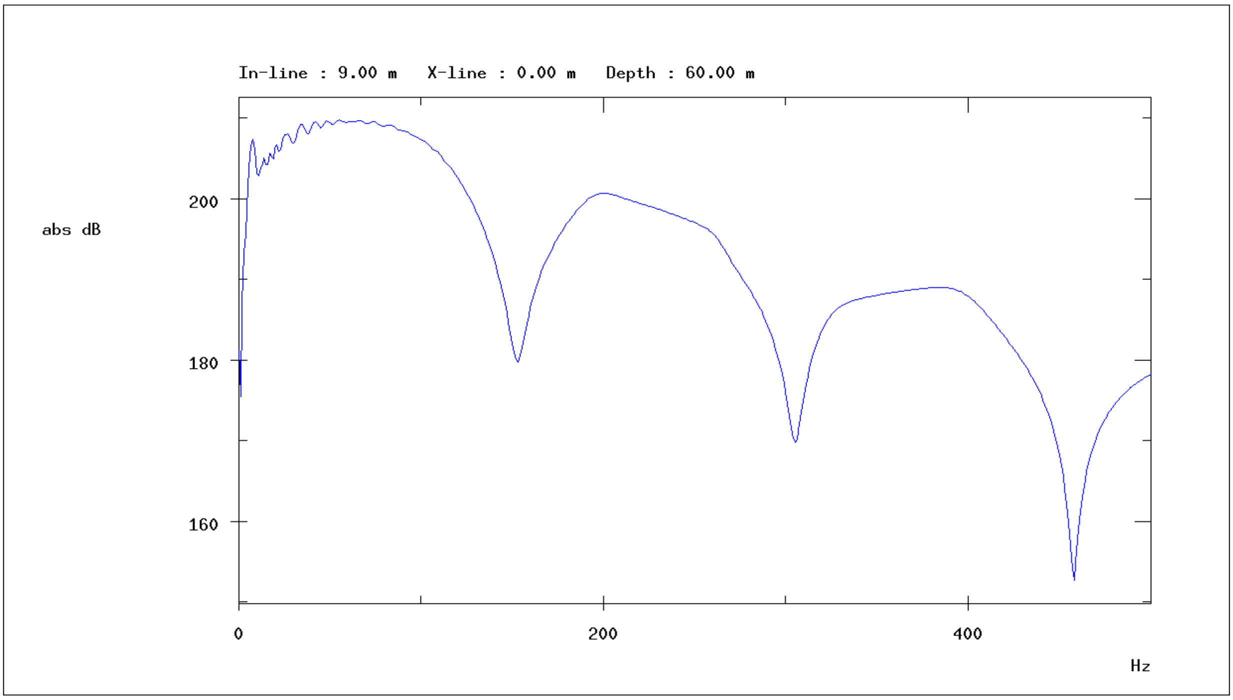

Figure 2.10. Amplitude-frequency spectrum of the near field pressure signature (Figure 2.9). Illustration: From Schoolmeesters, 2002, with permission.

2.6.1 Tracklines and shooting area

The shooting was confined to an area of 10 x 10 km with centre at the position of the experimental cages (Figure 2.11). The shooting was done along lines 10 km long, with courses 45°/225°. Distance between lines was 300 m, and the number of lines was 33. The lines close to the cages were adjusted to avoid too close passage of the ropes and buoys connected to the cages. The seismic shooting started in the northern corner of the seismic field on May 13 at 12:30, and lasted until May 15 at 20:13. The shooting order of the tracklines is shown in Table 2.3.

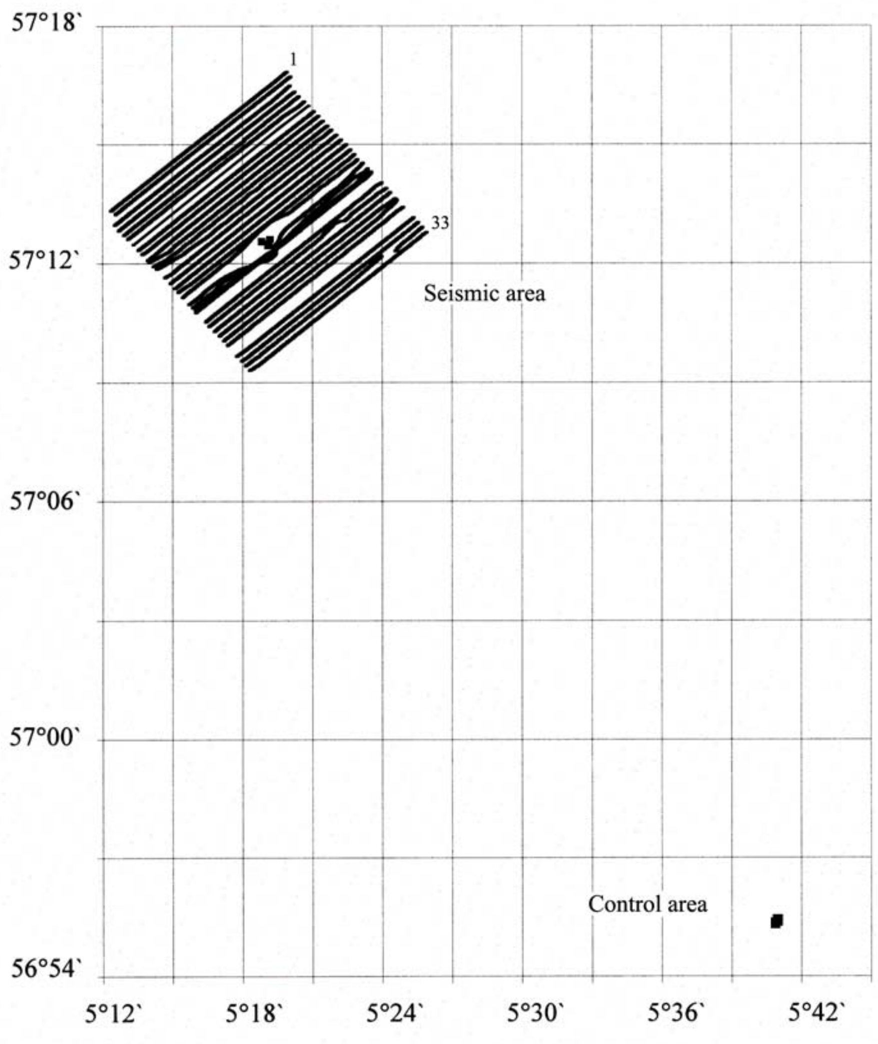

Figure 2.11. Tracklines and cages (squares) in the seismic- and control area. The shooting started in the northern corner, marked with line 1.

Table 2.3. Shooting sequence of the seismic lines. The survey was performed with soft-start procedure. Data from M/V "Falcon Explorer".

Sequence	Line ID	Date	Soft-start	Full volume	End of line
1	Fishoot01	13.05.2002	12:09	12:30	13:43
2	Fishoot07	13.05.2002	13:55	14:10	15:20
3	Fishoot02	13.05.2002	15:33	15:48	16:56
4	Fishoot08	13.05.2002	17:10	17:25	18:36
5	Fishoot03	13.05.2002	18:45	19:02	20:18
6	Fishoot09	13.05.2002	20:30	20:45	21:55
7	Fishoot04	13.05.2002	21:00	21:15	23:36
8	Fishoot10	13.05.2002	23:45	23:57	01:12
9	Fishoot05	14.05.2002	01:20	01:40	02:53
10	Fishoot11	14.05.2002	03:09	03:21	04:35
11	Fishoot06	14.05.2002	04:49	05:01	06:15
12	Fishoot12	14.05.2002	06:42	06:46	07:45
13	Fishoot18	14.05.2002	08:19	08:19	09:23
14	Fishoot13	14.05.2002	10:02	10:19	11:23
15	Fishoot19	14.05.2002	11:41	11:50	13:05
16	Fishoot14	14.05.2002	13:14	13:29	14:43
17	Fishoot20	14.05.2002	14:53	15:10	16:22
18	Fishoot33	14.05.2002	16:45	17:00	18:16
19	Fishoot28	14.05.2002	18:26	18:43	19:59
20	Fishoot32	14.05.2002	20:10	20:30	21:44
21	Fishoot27	14.05.2002	21:59	22:12	23:30
22	Fishoot31	14.05.2002	23:54	23:59	01:09
23	Fishoot26	15.05.2002	01:26	01:35	02:51
24	Fishoot30	15.05.2002	03:10	03:17	04:33
25	Fishoot25	15.05.2002	04:46	04:56	06:13
26	Fishoot29	15.05.2002	06:30	06:39	05:53
27	Fishoot24	15.05.2002	08:09	08:19	09:35
28	Fishoot17	15.05.2002	10:00	10:10	11:25
29	Fishoot23	15.05.2002	11:44	11:59	13:14
30	Fishoot16	15.05.2002	13:24	13:38	14:58
31	Fishoot22	15.05.2002	15:10	15:25	16:40
32	Fishoot15	15.05.2002	17:03	17:18	18:29
33	Fishoot21	15.05.2002	18:39	18:57	20:13

2.7 Statistics

Statistical analyses were done in STATISTICA 6.0 (Statsoft, Inc. 1984-2001).

When comparing the tailbeat frequency of the groups before, during and after shooting, Kruskal-Wallis ANOVA by ranks detected significant differences between the groups, and three individual Mann-Whitney U-tests were performed. To minimize type I errors the level of significance was adjusted by a Bonferroni correction ($^{\alpha}/_{k}$, where k is the number of comparisons), giving $\alpha = 0.0167$.

3 Results

3.1 Caged fish

All fish collected from the cages were alive, and almost all in the sandbox were dead. Only 5 living fish were collected in the sandbox (3 in cage 1, 1 in cage 2 and 1 in cage 3a). The number of dead and alive fish can be seen in Table 3.1. Some fish were impossible to measure, as the decaying process had gone too far.

Table 3.1 Soak time, number and mean length of the fish for each cage. SD = standard deviation.

Cage no.	Cage	Soak time [hours and minutes]	No. of sandeel	No. alive	No. dead	Mortality [%]	Mean length ± SD [cm]	No. of measured fish
1	Seismic	351 h 42 m	154	106	48	31.2	17.9 ± 2.5	130
2	Seismic	349 h 18 m	114	57	57	50.0	18.1 ± 2.6	90
3b	Seismic	177 h 0 m	69	56	13	18.8	18.1 ± 2.5	67
3a	Control*	154 h 42 m	378	243	135	35.7	17.2 ± 2.2	378
4	Control	160 h 36 m	32	20	12	37.5	13.3 ± 1.4	31
5	Control	159 h 7 m	51	31	20	39.2	13.4 ± 1.4	50

*Cage was placed in the seismic area, but was retrieved before shooting.

There were great differences in the number of caged fish and soak time among the cages. The differences in soak time were caused by the technical problems with cage 3a, which resulted in retrieval of the cage and deployment of cage 3b, and because cage 4 and 5 were deployed 8 days after the other cages due to problems finding high enough concentrations of fish. The fish in cages from the control area (cage 4 and 5) were significantly smaller than the fish caged in the experimental area (one-way ANOVA, *post-hoc* confirmation with Newman-Keuls test, $P < 0.0001$).

Due to unequal treatment (fish caught by trawling and shorter soak time) between the fish in cage 4 and 5 and the rest of the fish, the mortality data from the control and experimental group were difficult to compare. However, a Mann-Whitney U-test showed that there was no significant difference in percent mortality between the areas ($P > 0.05$), but when normalizing percent mortality by soak time we found significantly lower mortality in the cages exposed to seismic signal than in the control cages (Mann-Whitney U-test, $P < 0.05$) (Table 3.2). The fish in cage 3a was treated in the same way as the fish in the seismic cages (the cage was also deployed in the seismic area), but the mortality were still significantly higher than amongst the caged fish exposed to seismic signal. Cage 3a had also the highest density of sandeel with almost 2.5 times more fish than in cage 1, which was the cage with the second highest density.

Table 3.2 Percent mortality normalized by soak time.

Cage no.	Cage	Soak time [hours]	Mortality [%]	Mortality/hour [%/h]
1	Seismic	351.7	31.2	0.09
2	Seismic	349.3	50.0	0.14
3b	Seismic	177.0	18.8	0.11
3a	Control*	154.7	35.7	0.23
4	Control	160.6	37.5	0.23
5	Control	159.1	39.2	0.25

*Cage was placed in the seismic area, but was retrieved before shooting.

3.2 Grab sampling

Catches of sandeels taken by grab in the experimental area before (7-13 May) and after (15-19 May) shooting are presented in Figure 3.1, and number of grab shots taken each night is listed in Table 3.3. Nineteen grab shots were taken before shooting, and 59 after shooting. There were no catches of dead sandeel in the grab shots.

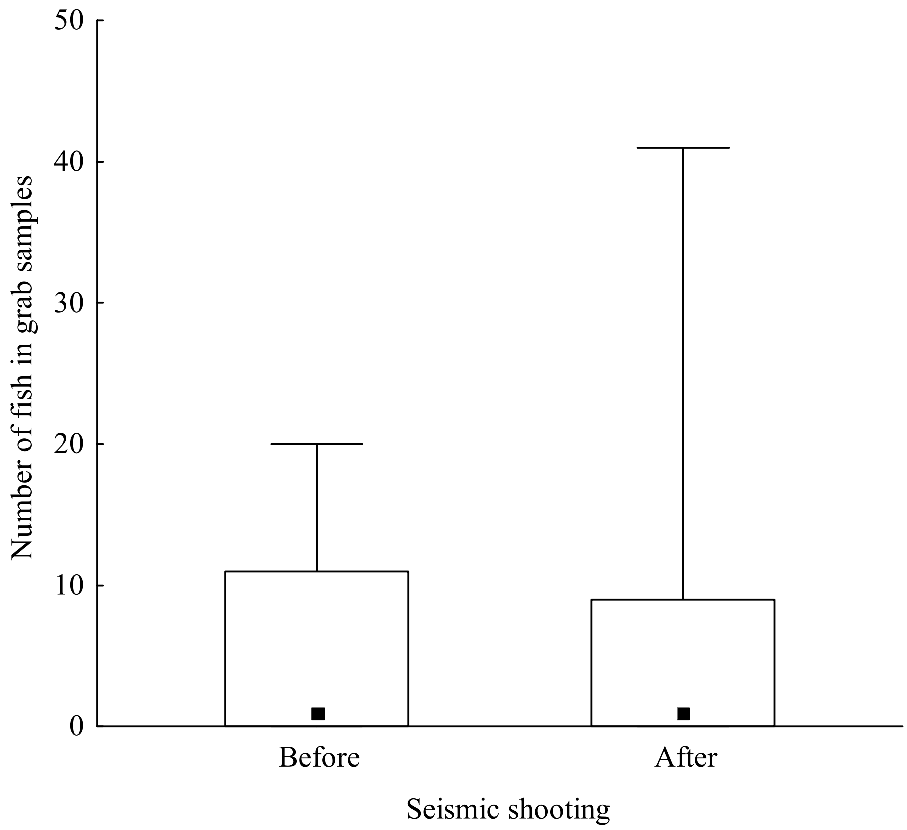

Figure 3.1. Grab samples before and after shooting. n_{Before}=19 and n_{After}=59.
■ Median, ☐ 25%-75% Quartiles, Min-Max.

The catches of sandeel showed no significant difference before and after shooting (Mann-Whitney U-test, $P > 0.05$), and the median value was 1 fish per sample in both periods.

Table 3.3. Number of grab shots taken each night, before and after the seismic shooting. The table also show the total catch of sandeels each night.

Seismic shooting	Date	Time	Grab shots	No. of sandeel
Before	07.05.2002	01:35-02:35	7	40
	11.05.2002	23:00-23:00	1	11
	12.05.2002	00:22-03:04	10	44
	13.05.2002	04:38-04:38	1	1
After	15.05.2002	22:45-23:45	2	31
	16.05.2002	01:00-03:40	15	94
	17.05.2002	01:07-03:05	14	85
	18.05.2002	00:17-02:11	14	77
	19.05.2002	00:10-01:58	14	39

3.3 Video analyses

The division of the "Aglantha videos" into 10 minutes time blocks resulted in 2 blocks before, 44 during and 16 after the shooting period. Most of the blocks were recorded from cage 1, but 11 blocks (A 1 c2 – A 11 c2, see Table 3.6) in the "after" category were recorded from cage 2. The total videolink recordings were approximately 86 hours with about 53 hours recorded in sufficient light conditions allowing analyses. The 53 hours were distributed on approximately 11 hours before, 29 hours during and 13 hours after the seismic shooting. Long play recordings with the timelapse recorder constituted approximately 11 hours in real observation time.

3.3.1 Vertical location of the sandeels in the cage

The "videolink videos" and the "timelapse videos" were analysed by freezing the picture every minute and the number of fish were counted. This resulted in a total of 3217 observations (695 before, 1744 during and 778 after shooting). The observations were normalized by hours by calculating the mean of every 60 observations, giving a new total of 55 observations (12 before, 30 during and 13 after shooting).

Due to variation in weather conditions, in particular in cloud cover, the period of sufficient light level in order to count the fish varied from day to day. There were generally acceptable observation conditions in the period 06:30-20:30. Number of observations each day and time period of the recordings are given in Table 3.4. One fish was seen entering the sand in the period before shooting, compared to 7 fish during and none after the shooting. No fish was seen to leave the sand before shooting, compared to 6 fish during and none after the shooting.

The majority of the sandeels stayed in the upper part of the cage before, during and after the seismic shooting. There was no significant difference in number of fish occupying the lower part of the cage between the three periods (Kruskal-Wallis ANOVA by ranks, $P > 0.05$) and the average number of fish was 0.09 before, 0.05 during and 0.03 after shooting (Figure 3.2).

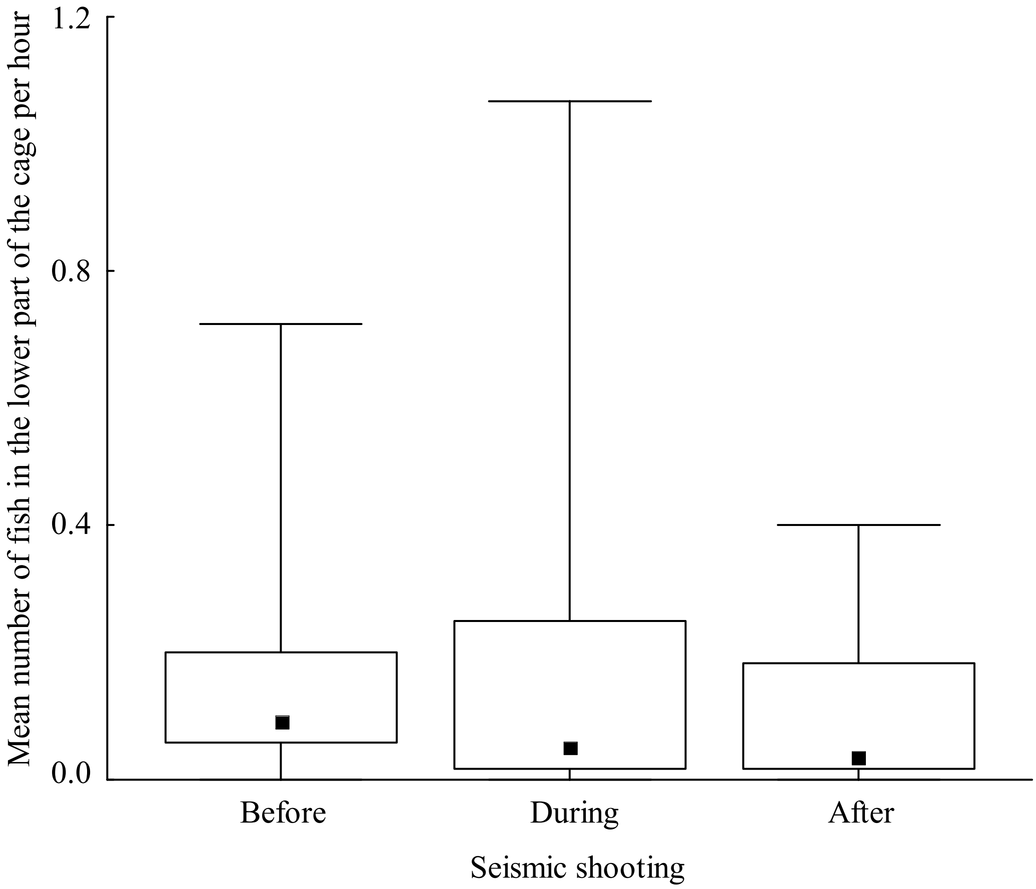

Figure 3.2. Mean number of fish registered in the lower part of the cage per hour during the experiment. $n_{Before}=12$, $n_{During}=30$ and $n_{After}=13$. ■ Median, ☐ 25%-75% Quartiles, ⌶ Min-Max.

Table 3.4. Number of observations each day and time of recordings.

Seismic shooting	Date	Time of recordings	No. of observations
Before	12.05.2002	06:30-08:20	111
	12.05.2002	12:35-20:45	491
	13.05.2002	10:57-12:29	93
During	13.05.2002	12:30-14:01	92
	13.05.2002	18:17-20:30	134
	14.05.2002	07:30-14:55	446
	14.05.2002	15:10-20:30	321
	15.05.2002	06:45-08:23	99
	15.05.2002	08:27-09:58	92
	15.05.2002	10:15-13:09	175
	15.05.2002	13:17-17:42	266
	15.05.2002	17:53-19:31	99
	15.05.2002	19:54-20:13	20
After	15.05.2002	20:13-20:45	32
	16.05.2002	06:45-08:05	81
	17.05.2002	06:30-08:22	113
	17.05.2002	08:51-13:02	252
	17.05.2002	20:25-21:30	66
	18.05.2002	05:45-08:37	173
	18.05.2002	10:40-11:40	61

3.3.2 C-start reactions

The C-start reaction started with a sudden jerk involving retraction of the head and tail (2), followed by a stage where the fish loosened up by straightening the tail (3) and ended with the fish swimming away (4), usually with increased speed and often in a different direction.

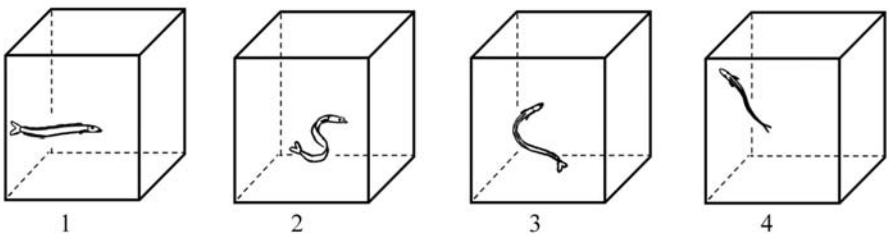

Figure 3.3. A general description of the C-start reaction. 1) Normal swimming, 2) sudden jerk with retraction of the head and tail 3) loosing up stage by straightening the tail 4) normal swimming, but with increased speed and changed direction. The cube represents a reference point.

The C-starts were seldom performed at the same time among the fish. Usually, there was only one fish reacting with C-start, but occasionally 2-3 fish reacted simultaneously. There were no observed C-starts before shooting. A total of 66 C-starts were observed during shooting and 1 after shooting. An overview of observation time and number of C-starts performed in relation to distance from tracklines are given in Table 3.5. The tracklines were shot continuously and the recordings from the cages did not cover all of them. The tracklines, on which the analysis was based, are also presented in Table 3.5. There was no significant correlation between the frequency of C-starts and the distance from tracklines ($r = -0.4054$, $P > 0.05$). There were significantly more C-starts observed during shooting compared to the other periods (Mann-Whitney U-test, $P < 0.05$).

Table 3.5. The number of C-starts performed before, during and after the seismic shooting and the frequency of C-starts performed based on minutes of observation time. The table also shows C-starts in relation to distance from tracklines. Note that the distance is estimated from the centre of the tracklines and only gives an approximate measure.

Seismic shooting	Trackline	Distance from centre of trackline to cages [km]	Observation time [minutes]	No. of C-starts	C-starts/ minute
Before			20	0	0.00
During	1	5.6	50	9	0.18
During	7	3.7	40	6	0.15
During	2	5.3	40	3	0.08
During	8	3.4	40	2	0.05
During	17	0.7	80	14	0.18
During	23	0.9	40	7	0.18
During	16	1.2	40	10	0.25
During	22	0.4	60	9	0.15
During	15	1.5	50	6	0.12
After			160	1	0.01

3.3.3 Tailbeat frequency

The counting of tailbeats from the 10 minutes time blocks resulted in a total of 620 observations (20 before, 440 during and 160 after shooting). A total 110 of the observations from the "after" category were recorded from cage 2 and thus excluded from the analyses. Hence, the analyses are based on 510 observations (20 before, 440 during and 50 after shooting). Number of observations each day and time period of the time blocks are given in Table 3.7 (see also Appendix chapter 6.1).

There were significantly differences in the tailbeat frequencies before, during and after shooting (Kruskal-Wallis ANOVA by ranks, $P < 0.0001$), and the median values for the three periods were 1.26 b/s, 1.98 b/s and 2.1 b/s, respectively (Figure 3.4). The median value for the not-included time blocks from cage 2 was 2.05 b/s after shooting.

The tailbeat frequency was significantly higher both during and after compared to before shooting (Mann-Whitney U-tests, Bonferroni adjusted $\alpha = 0.0167$, $P < 0.0001$ for both tests). There was no significant difference between during and after shooting (Mann-Whitney U-test, Bonferroni adjusted $\alpha = 0.0167$, $P > 0.05$).

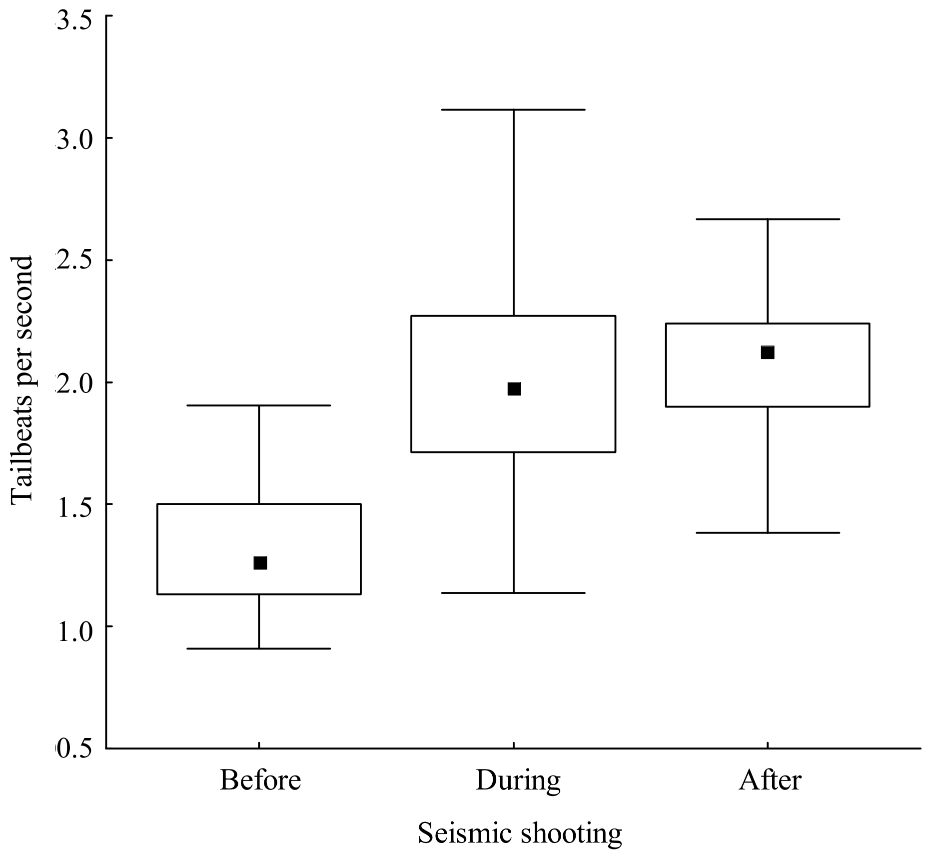

Figure 3.4. Tailbeats per second before, during and after shooting. $n_{Before}=20$, $n_{During}=440$ and $n_{After}=50$. ■ Median, 25%-75% Quartiles, Min-Max.

The tailbeat frequency increased during the days of shooting and did not return to preshooting levels during the 3-day period after shooting ended. Box plots of the frequencies on the different days are given in Figure 3.5.

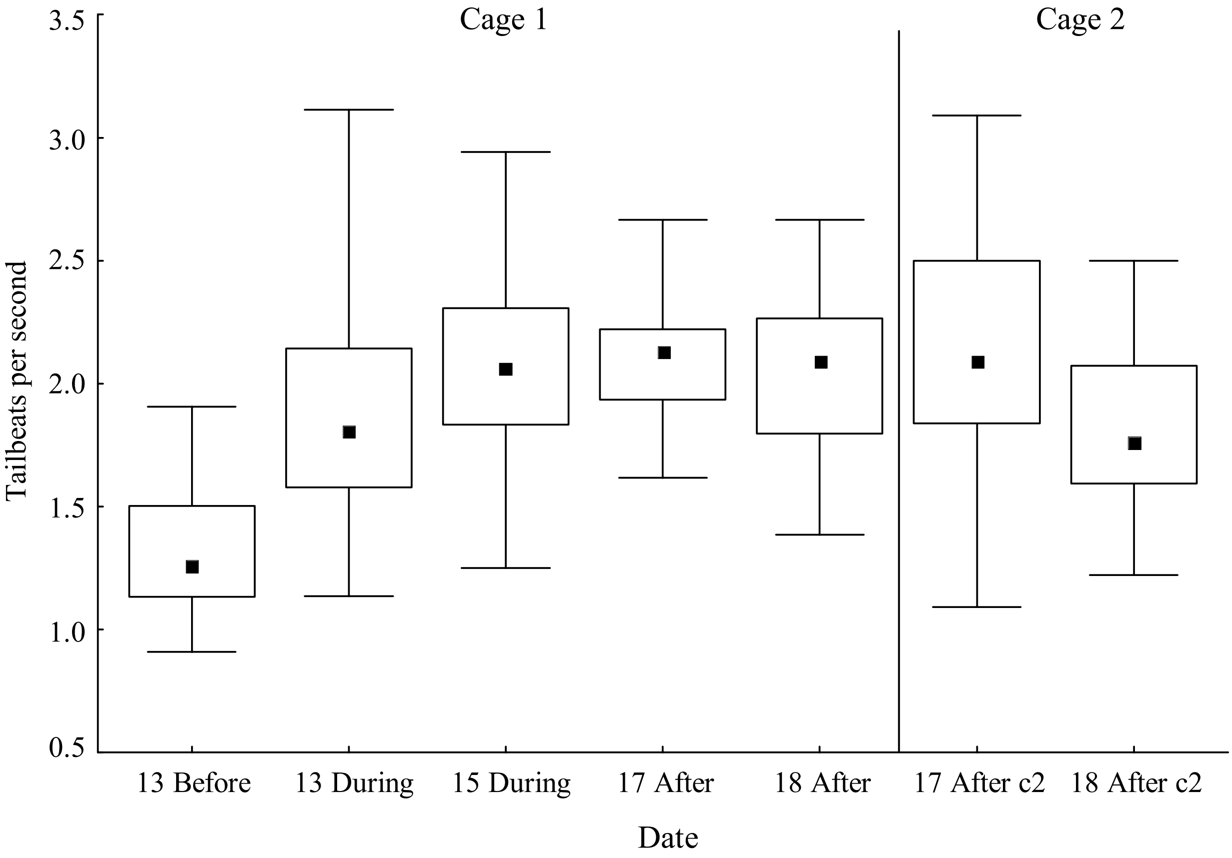

Figure 3.5. Tailbeats per second on the different days. The figure also shows frequencies for the fish in cage 2. The numbers on the x-axes represent dates. $n_{13Before}$=20, $n_{13During}$=170, $n_{15During}$=270, $n_{17After}$=30, $n_{18After}$=20, $n_{17After\ c2}$=90 and $n_{18After\ c2}$=20. ■ Median, 25%-75% Quartiles, Min-Max.

Table 3.7. Number of observations each day and time periods of recordings. B = before shooting, D = during shooting, A = after shooting and A c2 = after shooting cage 2. The time blocks are given in details in the appendix chapter 6.1, Table 6.1.

Seismic shooting	Date	Time of recordings	Time blocks	Cage	No. of observations
Before	13.05.2002	12:10:00 - 12:30:00	B 1 - B 2	1	20
During	13.05.2002	12:46:30 - 13:26:30	D 3 - D 6	1	40
	13.05.2002	14:06:00 - 14:36:00	D 7 - D 9	1	30
	13.05.2002	14:43:00 - 14:53:00	D 10	1	10
	13.05.2002	15:15:30 - 15:25:30	D 11	1	10
	13.05.2002	15:45:30 - 16:15:30	D 12 - D 14	1	30
	13.05.2002	16:54:00 - 17:04:00	D 15 - D 16	1	10
	13.05.2002	17:31:00 - 17:51:00	D 17	1	20
	13.05.2002	18:01:00 - 18:11.00	D 18	1	10
	13.05.2002	18:40:00 - 18:50:00	D 19	1	10
	15.05.2002	10:13.30 - 10:43:30	D 20 - D 22	1	30
	15.05.2002	10:46:00 - 11:36:00	D 23 - D 27	1	50
	15.05.2002	11:56:00 - 12:16:00	D 28 - D 29	1	20
	15.05.2002	12:34:00 - 12:44:00	D 30	1	10
	15.05.2002	13:10:00 - 13:20:00	D 31	1	10
	15.05.2002	14:02:00 - 14:12:00	D 32	1	10
	15.05.2002	14:30:00 - 15:00:00	D 33 - D 35	1	30
	15.05.2002	15:44:00 - 16:44:00	D 36 - D 41	1	60
	15.05.2002	17:24:00 - 17:44:00	D 42 - D 43	1	20
	15.05.2002	17:55:00 - 18:25:00	D 44 - D 46	1	30
After	17.05.2002	09:39:00 - 10:09:00	A 47 - A 49	1	30
	17.05.2002	11:00:00 - 11:30:00	A 1 c2 - A 3 c2	2	30
	17.05.2002	11:37:05 - 12:37:05	A 4 c2 - A 9 c2	2	60
	18.05.2002	09:59:00 - 10:19:00	A 50 - A 51	1	20
	18.05.2002	10:54:10 - 11:14:10	A 10 c2 - A 11 c2	2	20

3.4 Other observations

When investigating the sandeels from the cages more closely, it was discovered that several fish had severe injuries on the mouthparts, and especially on the protruding lower jaw (mandibles). By further examination of the videos it was observed that sandeels sometimes “hang” with the mouth in the meshes. The injuries were seen on fish from all cages (seismic area and control area), but unfortunately, injured control fish were not counted. The proportion of injured fish from the experimental area was: 20.8 % in cage 1, 26.3 % in cage 2 and 10.0 % in cage 3b.

During shooting of line 16 on 15 May (13:00-14:00) schools of sandeel were observed swimming above the ROV. This observation was done in the experimental area with the top-camera on the ROV.

On the same day at 19:52, 21 minutes before the shooting finished, a group of 10-15 free-living sandeels was observed in the sand, and fled out of the sand when the ROV approached. This was in the experimental area and just outside cage 3b.

Around 09:00 on 17 May a group of white-beaked dolphins (*Lagenorhynchus albirostris*) was observed in the experimental area, and at least two of them stayed around the vessel until 19 May, when the experiment ended.

4 Discussion

Our results suggest that seismic shooting has no immediate lethal effects on the lesser sandeel and that such shooting only has a moderate effect on the sandeel's behaviour. The mortality in the cages was probably a result of injuries due to handling and confinement and not caused by seismic shooting. This is supported by the fact that there was no dead sandeel in the catches taken in grab and the catches were on average equal both prior to and after shooting. Contrary to the fishermen's statement the caged sandeel preferred to stay in the upper part of the cage during all three time periods, and there was no indication that the sandeels fled into the sand when exposed to the shooting. However, seismic shooting seemed to elicit C-start responses and to increase the sandeel's tailbeat frequency. Almost all C-starts were recorded during seismic shooting, none was recorded before and only one was recorded after. The tailbeat frequency was significantly higher both during and after than prior to seismic shooting and did not return to preshooting levels during the 3-day period after the shooting.

4.1 Material and methods

To the best of our knowledge this is the first study to examine effects of seismic shooting on fish by using standard 3D seismic survey as sound source and *in situ* video observations of the exposed fish. In studies on seismic shooting and fish behaviour with the aid of either *in situ* video (Wardle *et al.*, 2001) or direct observations (Pearson *et al.*, 1992), the sound pressure level (SPL) corresponded to ordinary 3D surveys, but the performance of the shooting procedure was often different. In an ordinary 3D survey the seismic vessel operates along tracklines, the duration of the shooting is often between 2.5 and 5 days and nowadays, the survey is usually performed with soft-start procedure. When operating along tracklines the sound level at any given location (e.g. at the cages) will increase as the vessel approaches and decrease as the vessel moves away. Also, in our experiment the order of the lines were shot in a non-increasing order. The first line that was shoot was number 1, then number 7, number 2, number 8 etc. (see Table 2.3). This means that the sound source approached the cages very slowly and that the sound level at the cages rose in a non-linear way.

Together with the soft-start procedure, this could have made the fish habituate to the sound to a larger extent than when air-guns are suddenly discharged in close vicinity to the fish. As a result we could expect reactions caused by seismic signals to diminish over time. However, such an effect was not clearly seen in this experiment and the C-starts seemed to happen randomly, and not in the beginning of the first line or of any other lines. The energy generated by air-gun arrays is concentrated vertically downwards (and vertically upwards), and the amplitude levels emitted horizontally will typically be about 20 dB lower than those emitted vertically. The fish was probably hit by horizontally emitted energy and energy that has been reflected from the seabed, sea surface or density layers in the sea (i.e. thermo- and halocline), as the vessel only moved around the cages and did not pass over them. This, together with the continuously moving vessel, would create a complex sound image that can hardly be copied by using stationary sound sources or simulated seismic surveys.

The cages were properly designed and functioned as expected. However, to catch sandeel it was necessary to hit aggregations of sandeels when the cages were lowered to the bottom. This worked in the experimental area, but not in the control area. When catching sandeel with this method it is not possible to control the number of fish caught, and this resulted in different fish densities in the cages. There were more than five times as many sandeels in cage 3a (378 specimens) as in 3b (69 specimens), and this may have affected the sandeels' behaviour and mortality rate. Cage 3a was the cage placed in the experimental area but was regarded as a control cage owing to the fact that it was retrieved before shooting, and the fish was treated the same way as the fish in the other experimental cages. Still, when normalizing percent mortality by soak time we found that the mortality was significantly higher in this cage compared to the other experimental cages. This may indicate too high fish density in cage 3a, but there could also be another explanation. Other studies using caged fish have shown that the majority of fish mortality occurs within the first 8 days of captivity (Sangster *et al.,* 1996) and this may be the reason why the mortality rate was higher in cage 3a compared to the cages placed in the same area. The soak time for cage 3a was 154.7 hours, whereas cage 1, 2 and 3b had soak times of 351.7, 349.3 and 177.0 hours, respectively. However, it is unknown if the high fish density had consequences on the behaviour since the majority of the video recordings done with the ROV were from cage 1 (the rest are from cage 2), and all other recordings with the SIT camera were from cage 3b.

Sandeel catches by grabs are well known (McIntyre, 1958; Petersen, 1977), and grab sampling has been suggested as a method for studying density of wintering sandeel (Høines and Bergstad, 2000). The grab sampling in this experiment worked out well, but as experienced in Høines and Bergstad (2000) sandeel were often observed with their head sticking out between the closed jaws of the grab. It is therefore likely that some sandeels escaped during capture, and that the catches only gave a minimum abundance estimate.

The set-up of the field experiment, and especially the design of the cages, was mainly based on the fishermen's statements that seismic shooting causes sandeel to flee to the bottom, burry deep in the sediment and die. The field experiment was also designed to determine whether the sandeels fled from the area where the seismic vessel operated. Although, the main goal was to investigate if these hypotheses could be verified, other reactions and behavioural changes could also be detected from the video recordings. However, the experimental set-up was not optimal for such extensive behavioural studies.

The SIT camera was mounted in the corner 2/3 up the wall pointing downwards and covered most of the bottom and walls. The intention was to monitor fish digging into and out of the sand and to see if the fish would aggregate in the lower part of the cage. As the fish preferred to stay in the upper part of the cage, a better position of the camera would have been hanging from the roof pointing downwards allowing more of the inside to be monitored. A minor problem was that under certain current conditions the walls started to move inwards into the cage and the visual range decreased. Again, this problem could have been solved if the camera were mounted in the roof and not in a corner.

The ROV used searchlight when navigating to the cages, but when the ROV was in position the light was switched off. Sandeels react to light (Winslade, 1974), and the light from the approaching ROV could have affected their behaviour. This was taken into account when making the time blocks and no time blocks were made when the light was on or within the first 10 minutes after it had been switched off. The ROV had slight positive buoyancy, and the top-thrusters had to be running to hold the ROV steady and close to the bottom. It is not known how much noise this made, but when comparing recordings from camera cage 3a with the ROV recordings, the sandeels seemed unaffected by this noise.

Carrying out large field experiments at this scale is time consuming and expensive, and consequently dependent on that everything works out with regard to time plans, weather conditions, equipments and as in this experiment a high density of fish in two areas. Lacking a proper control, due to technical problems and unsuccessful catch of fish, is of course sub-optimal and it may be argued that our results are not conclusive.

One major problem is how the cages might have influenced on the sandeel's behaviour. Caged fish presumably behave differently from free-living fish, and the effects of being caged (i.e. stress for instance) will probably be high with no acclimatization period. With proper control cages these effects could have been be ruled out, but unfortunately the control fish were treated differently and the observation time of the control cages was short.

There were indications that the cages stressed the fish and disturbed their diel rhythm. Sandeels were observed lying buried in the sand in the experimental area on the last day of shooting at 19:52. At the same time in the camera cage, there was not observed any sandeel entering the sand. During the experiment sandeels were not observed to come out of the sand at dawn. This means that the sandeels either entered and left the sand when it was too dark to observe them or that they stayed in the water column throughout the night. In the latter case diel rhythm seems severely disturbed by the cages.

The cages could also have affected other aspects of the sandeel's behaviour, and the question is if the sandeel would have fled into the sand if they were uncaged. The observation of a school of free-living sandeels swimming above the ROV after two days of shooting suggests that this is not the case, and give some indications that sandeels do not flee into the sand when exposed to seismic activity.

4.2 Results

The grab sampling showed equal catches (median = 1) of sandeels before and after the seismic survey. This indicates that sandeels did not moved out of the area during shooting. It is unlikely that the sandeels left the area when the shooting began and returned immediately after shooting ended. The shooting ended on 15. May at 20:13 and the first grab shot in the after-period was made only 2 hours and 32 minutes later. This grab shot caught 8 specimens. If the sandeels had left the area during shooting, the catches shortly afterwards should been expected to be smaller. It cannot be ruled out that the sandeel could have made short escapements as the seismic vessel approached, just to return to their original place after the vessel passed by. However, such short escapements would probably have minor consequences both for fish and fisheries.

The van Veen grab only sampled the top-layer of the sediment, not deeper than about 10 cm, and there are therefore no indications that the sandeels buried deeper after the seismic shooting, since the average catch rate was equal before and after shooting.

The mortality was on average about 35 % both in the experimental and control groups, but when normalizing percent mortality by soak time we found significantly lower mortality in the cages from the experimental area. The reason to this difference was most likely the treatment of the fish from the control area (trawled fish placed in buckets and released into the cages) and the fact that the fish were significantly smaller than the fish from the experimental area. The differences can also be explained by a high mortality in the early stages of captivity. The high mortality in cage 3a was could also be connected high fish density. The overall fish mortality was most likely caused by other factors than seismic activity, like injuries from contact with the net.

The analyses of the location of sandeel in the cage showed that the sandeels stayed in the upper part of the cage before, during and after the seismic shooting. This analysis was based on the assumption that all fish stayed above the SIT camera and none was hiding in the sand. This assumption was verified by several observations. Every time the ROV inspected the cages the sandeels were observed to stay in the upper part, above the SIT camera and very close to the roof in all cages. This might be due to the ROV's presence, but there are SIT camera recordings when the ROV inspected cage 3b showing that the sandeels neither emerged or fled into the sand, neither before, during or after the inspecting ROV. The possibility still remains that one or two individuals could have hided in the sand and missed out from the recordings. The observations of more fish digging in than out of the sand indicate that this might have taken place. All the same, these observations are few compared to the total number of fish being caged and the majority of fish clearly stayed in the upper part of the cage.

The preference for the upper part of the cages may be related to different water flow in different parts of the cage. It is well known that the current strength is lower close to the bottom than higher up in the water column. A stronger current in the upper part of the cage would have brought more oxygen and possibly also more food particles through the meshes than in the lower part of the cage. The 0.3 m high base frame surrounding the bottom could have led to an even weaker current just above the bottom.

The analyses of the time blocks showed that several fish performed C-starts during the seismic shooting, whereas only one C-start was registered after the shooting finished and there is therefore reason to believe that the sandeels performed C-starts as a consequence of the air-gun discharges. After the C-start reaction the sandeels resumed normal swimming behaviour, but often in a different direction. Blaxter *et al.* (1981) found that herring almost always showed directional responses away from the source, whereas Wardle *et al.* (2001) found that saithe fled away from the air-gun discharge only when the explosion was visible. When not visible, they kept swimming unaltered towards the air-gun. It is difficult to tell if the responses of the sandeels were directional with respect to the sound source, since the sound image in the cage was not known. The C-start response in sandeel appeared to be somewhat different from other teleosts (e.g. gadoids), and this is probably related to differences in body shape and swimming patterns. The sandeel has a very slender body and an eel-like swimming pattern, and the lack of swimbladder forces them to swim constantly. This could be the reason why the sandeel's C-starts seem more "acrobatic" than the corresponding behaviour in other teleosts. There has not been published any works on C-starts in sandeel, but Eaton *et al.* (1977) did some work on the spiny eel (*Caecomastacembelus loennbergi*), a species with a similar body shape as sandeels, and found that the spiny-eel reacted to a vibrational stimulus by retracting its head. A similar reaction was seen in stage 2 (Figure 3.3) of the C-start performance of the sandeels.

The tailbeat analyses showed a significant increase in tailbeat frequency both during and after shooting compared to before shooting, and the tailbeat frequency did not return to preshooting levels during the 3-day period after shooting. This indicates that the seismic shooting affected the sandeels activity level and that the effect lasted for more than three days. The analysis showed a rapid increase of tailbeat per second when comparing the time blocks from before shooting to the time blocks from when the shooting just had begun. The two time blocks before shooting had mean tailbeats of 1.3 b/s and 1.4 b/s while the two time blocks when the shooting had just started (16.5 minutes after the "before" blocks) both had means of 1.8 b/s. These "before" blocks had also the lowest means of all time blocks made from the recordings (see also Appendix chapter 6.1.).

A possible criticism of the results from the tailbeat frequency analysis and the vertical location analysis is the use of time dependent data. The observations from both analyses were dependent with regards to time, but were treated as independent. In an attempt to minimize this dependency the observations from the location analysis were normalized by calculating the means for every hour and in the tailbeat frequency analysis a strong effort was made to avoid counting the same fish several times. These actions decrease the data dependency but will not turn them into independent data. Still, it is unlikely that that the dependent data could have caused the results obtained and the conclusions to be flawed.

The observation of a group of white-beaked dolphins swimming in the experimental two days after shooting ended can be proposed to have affected the results. The white-beaked dolphins are, like other dolphins, fish-eaters, with the lesser sandeel as common prey (Reeves *et al.*, 1999), and it is likely that if the sandeels had sensed the dolphins' presences they could have been scared and so on affected the results. It has been suggested that some clupeid fishes can detect echolocating cetaceans (Mann *et al.*, 1997), but since the sandeel's ear structure are very different from the clupeiformes, it is unlikely that sandeels have the same ability. Another option is that the sandeels could have sensed the whales visually, and there is one observation indicating that this might have happened. On the 17 May at about 07:17 just 2 hours before the dolphins were discovered it was observed 15-20 sandeels entering the lower part of the cage simultaneously. This reaction was counted as 9 specimens being in the lower part because all counts were done at whole minutes, but it was still a very high number compared to the other observations (the next highest was 4). Anyway, it did not influence the location results in a major way, since the number of observations was high, and we believe that this was the only possible reaction caused by the whales.

Why does it seem like the sandeel are less influenced by seismic signals compared other species? To explain this it is important to first consider what separates this study from other seismic studies. As mentioned earlier, this study seems to be the first to combine *in situ* observations of fish in relation to standard 3D seismic survey, but also the first to be conducted on a species lacking swimbladder. It is well known that the swimbladder plays a key role in hearing (Hawkins, 1993), and a lack of swimbladder will probably raise the sandeel's hearing threshold and thus contribute to poorer hearing abilities than among species having swimbladder. For example, the dab, another species lacking swimbladder, has a hearing threshold of about 92 dB re 1 μPa (Chapman and Sand, 1974; Hawkins, 1993), which is 12 dB above the hearing threshold of cod (80 dB re 1 μPa, Chapman and Hawkins, 1973; Hawkins, 1993). An increase of 12 dB in the hearing threshold means that the sound pressure has to be 4 times higher to reach the threshold for dab than for cod. It is likely to believe that the same conditions applies for sandeel and that a poor hearing ability makes sandeel less influenced by noise compared to species having swimbladder.

Having mentioned these main differences, which separate this study from others, we begin to understand that this is pioneer work in a research field far from fully explored. Not much work has been done on this subject and it is hard to find any replicate studies. The majority of the studies have shown that seismic shooting has a negative impact on catch rates and abundance of several fish species (rockfish, cod and haddock, Skalski *et al.,* 1992; Løkkeborg and Soldal, 1993; Engås *et al.,* 1996) and that such shooting also affects their behaviour (rockfish, saithe and haddock, Pearson *et al.*, 1992; Wardle *et al.,* 2001) However, there are also studies, with the opposite conclusion suggesting that seismic shooting has little or no effects on fish (rainbow trout (*Salmo gairdneri*) and salmon (*Salmo salar*), Thomsen, 2002) and fisheries (Jákupsstovu *et al.,* 2001). This implies that the subject is difficult and complex, and that there is need for replicate studies. It is recommendable that future studies use standard seismic surveys in an attempt to get the most realistic results.

Due to this complex nature of the subject it is also important to apply a precaution approach. The lesser sandeel seems to be a robust species in this connection that are affected by seismic shooting but not in a serious or lethal way. However, it must be stated that this field study does not provide any answers regarding potential long-term effects caused by seismic shooting. McCauley *et al.* (2003) found for instance that high intensity anthropogenic sound damages fish ears in an experiment mimicking the sound stimulus from a passing seismic vessel. As in our experiment, the fish were caged and he found indications that the fish would have fled from the sound source if they were free. But there have been done very little research on such effects, and it cannot be excluded that this could have happened to the lesser sandeel, also if they were free.

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6 Appendix

6.1 Appendix I

Table 6.1. Detailed list of time blocks and mean tailbeats per time block.

No.	Seismic shooting	Day	Time block	Track-line	Time	Mean tailbeats per time block	Cassette
1	Before	13	B 1		12:10:00 - 12:20:00	1,267	4
2	Before	13	B 2		12:20:00 - 12:30:00	1,403	4
3	During	13	D 3	1	12:46:30 - 12:56:30	1,786	4
4	During	13	D 4	1	12:56:30 - 13:06:30	1,802	4
5	During	13	D 5	1	13:06:30 - 13:16:30	2,020	4
6	During	13	D 6	1	13:16:30 - 13:26:30	1,681	4
7	During	13	D 7	1	14:06:00 - 14:16:00	1,640	4
8	During	13	D 8	7	14:16:00 - 14:26:00	1,872	4
9	During	13	D 9	7	14:26:00 - 14:36:00	1,723	4
10	During	13	D 10	7	14:43:00 - 14:53:00	1,779	5
11	During	13	D 11	7	15:15:30 - 15:25:30	1,807	5
12	During	13	D 12	2	15:45:30 - 15:55:30	1,720	5
13	During	13	D 13	2	15:55:30 - 16:05:30	1,777	5
14	During	13	D 14	2	16:05:30 - 16:15:30	1,751	5
15	During	13	D 15	2	16:54:00 - 17:04:00	2,465	5
16	During	13	D 16	8	17:31:00 - 17:41:00	1,977	5
17	During	13	D 17	8	17:41:00 - 17:51:00	2,062	5
18	During	13	D 18	8	18:01:00 - 18:11:00	2,050	5
19	During	13	D 19	8	18:40:00 - 18:50:00	1,944	5
20	During	15	D 20	17	10:13:30 - 10:23:30	1,884	6
21	During	15	D 21	17	10:23:33 - 10:33:30	1,912	6
22	During	15	D 22	17	10:33:30 - 10:43:30	2,206	6
23	During	15	D 23	17	10:46:00 - 10:56:00	1,739	6
24	During	15	D 24	17	10:56:00 - 11:06:00	2,107	6
25	During	15	D 25	17	11:06:00 - 11:16:00	1,986	6
26	During	15	D 26	17	11:16:00 - 11:26:00	2,230	6
27	During	15	D 27	17	11:26:00 - 11:36:00	2,039	6
28	During	15	D 28	23	11:56:00 - 12:06:00	2,216	6
29	During	15	D 29	23	12:06:00 - 12:16:00	1,885	6
30	During	15	D 30	23	12:34:00 - 12:44:00	1,962	6
31	During	15	D 31	23	13:10:00 - 13:20:00	2,028	6
32	During	15	D 32	16	14:02:00 - 14:12:00	2,047	7
33	During	15	D 33	16	14:30:00 - 14:40:00	1,832	7
34	During	15	D 34	16	14:40:00 - 14:50:00	2,174	7
35	During	15	D 35	16	14:50:00 - 15:00:00	2,240	7
36	During	15	D 36	22	15:44:00 - 15:54:00	2,185	7
37	During	15	D 37	22	15:54:00 - 16:04:00	1,849	7
38	During	15	D 38	22	16:04:00 - 16:14:00	1,995	7
39	During	15	D 39	22	16:14:00 - 16:24:00	2,151	7
40	During	15	D 40	22	16:24:00 - 16:34:00	2,279	7
41	During	15	D 41	22	16:34:00 - 16:44:00	2,151	7
42	During	15	D 42	15	17:24:00 - 17:34:00	2,310	7
43	During	15	D 43	15	17:34:00 - 17:44:00	2,306	7
44	During	15	D 44	15	17:55:00 - 18:05:00	2,203	8
45	During	15	D 45	15	18:05:00 - 18:15:00	2,243	8
46	During	15	D 46	15	18:15:00 - 18:25:00	2,009	8
47	After	17	A 47		09:39:00 - 09:49:00	2,100	8
48	After	17	A 48		09:49:00 - 09:59:00	2,245	8
49	After	17	A 49		09:59:00 - 10:09:00	2,009	8
50	After cage 2	17	A 1 c2		11:00:00 - 11:10:00	2,206	8
51	After cage 2	17	A 2 c2		11:10:00 - 11:20:00	2,241	8
52	After cage 2	17	A 3 c2		11:20:00 - 11:30:00	1,880	8
53	After cage 2	17	A 4 c2		11:37:05 - 11:47:05	2,004	9
54	After cage 2	17	A 5 c2		11:47:05- 11:57:05	2,074	9
55	After cage 2	17	A 6 c2		11:57:05 - 12:07:05	2,223	9
56	After cage 2	17	A 7 c2		12:07:05 - 12:17:05	2,178	9
57	After cage 2	17	A 8 c2		12:17:05 - 12:27:05	2,286	9
58	After cage 2	17	A 9 c2		12:27:05 - 12:37:05	2,246	9
59	After	18	A 50		09:59:00 - 10:09:00	1,983	9
60	After	18	A 51		10:09:00 - 10:19:00	2,148	9
61	After cage 2	18	A 10 c2		10:54:10 - 11:04:10	1,870	9
62	After cage 2	18	A 11 c2		11:04:10 - 11:14:10	1,779	9