The effect of pulse stimulation on biota – Research in relation to ICES advice – Progress report on the effects on cod.

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Contents

Assignment

The work has been commissioned by the authority Directorate of Fisheries of the Ministry of Agriculture, Nature and Food Quality of the Netherlands and conducted in cooperation with the private company Verburg-Holland Ltd. of Colijnsplaat, the Netherlands.

Confidentiality

Details of the pulse trawl system developed by Verburg-Holland Ltd. and in particular the characteristics of the stimulus are kept confidential and therefore not revealed in this report. The research agreed with IMR Bergen was commissioned by contract which included a confidentiality paragraph.

Quality Assurance

IMARES utilises an ISO 9001:2000 certified quality management system (certificate number: 08602-2004-AQ-ROT-RvA). This certificate is valid until 15 December 2009. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. The last certification inspection was held the 16-22 of May 2007. Furthermore, the chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2000 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2009 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation, with the last inspection being held on the 12th of June 2007.

It is foreseen to involve members of the Expert Group on Pulse Trawling, who worked on the ICES Advice, to give guidance on the proper methodology applied. In addition publishing articles in peer-reviewed magazines provides an opportunity for international critique and quality testing.

Summary

In response to questions asked by ICES on the effects of pulse stimulation in commercial beam trawling on components of the marine ecosystem a number of preliminary studies were undertaken in the period between 1 September 2008 and 01 March 2009.

These activities involved the exposure of cod to a simulated electric pulse under laboratory conditions and observation of behaviour, including the foraging response, and monitoring mortality and possible internal injuries such as vertebral damage by X-ray photography. The research was conducted in cooperation with the Institute of Marine Research Bergen, IMR research station, Austevoll. This research station facilitated the research, target fish which were derived from their own aquaculture research stock and video observation equipment. The electric pulse simulator was made available by Verburg-Holland Ltd. with pulse characteristics similar to the commercial Verburg pulse system.

Groups of 20 fishes with similar lengths (0.41 – 0.55 m) were exposed to the electric stimulus, with each group in one of three distance ranges:

1. A “far field” range with the fish exposed at 0.4 m side ways of a conductor element.
2. A “above field” range with the fish exposed at 0.2-0.3 m above the centre of a conductor pair;
3. A “near field” range with the fish exposed at 0.1 m from the conductor element;

To exclude the effects of transfer and other unknown influences a control group of 20 fish was confined in the same way, but not exposed to the electric stimulus.

The fish exposed in the “far field” range, representing the fish just outside the working range of the trawl, showed hardly a reaction to the exposures and responded normally to the feeding cycles. The fish exposed in the “above field” range showed a moderate contraction of the muscles, but all recovered well and responded normally to the feeding cycles. The effects on the fish exposed in the “near field” range were more pronounced, 4 fishes died shortly after the exposure, and another 2 died in the observation period thereafter. In the observed period of 14 days after the exposures the surviving fish packed together outside the feeding zone and hardly responded to the feeding cycles.

The fish of the control group, exposed to a similar treatment as the exposed groups except receiving the pulse stimulation, showed a decrease in appetite compared to the fish exposed in the “far field” and “above field” ranges. This could have been related to the fact that this group was treated towards the end of the experimental period and thus stayed the longest time in the transfer tank.

Post mortal analysis using X-ray scans revealed that 5 out of 16 remaining fishes exposed in the “near field” range had hemorrhages close to the vertebral column, and of these five, 4 had vertebral bone fractures. No injuries were found on the fish exposed in the “above field” range, that showed weaker reactions to the electric exposure.

1. Introduction

In response to ecosystem related concerns about bottom trawling and particularly beam trawling that were raised by various scientists in the last decades of the previous century (Anon., 1988, 1995; Jennings and Kaiser, 1998; Lindeboom and De Groot, 1998; Kaiser and De Groot, 2000; Paschen *et al.*, 2000; Fonteyne and Polet; 2002; Piet *et al.*, 2000) pulse stimulation was developed as an alternative to tickler chains to enable the catch of flat fish, in particular sole (*Solea vulgaris* L.) and plaice (*Pleuronectes platessa* L.). Many studies were done in the 1970s and 1980s, but in spite of promising results commercial uptake was lacking (De Groot and Boonstra, 1970, 1974; Vanden Broucke, 1973; Stewart, 1975, 1978; Horn, 1976; Horton, 1984; Agricola, 1985; Van Marlen et al., 1997). The development of pulse trawling was again taken up in the 1990s by a private company (Verburg-Holland Ltd.) in The Netherlands (Van Marlen, *et al.*, 1999; 2000; 2001a, b). This led to trials over a complete year on a commercial vessel fully equipped with the new technology (Van Marlen, *et al.*, 2000; 2005a, b; 2006).

Meanwhile questions about ecosystem effects of introducing pulse beam trawling in the Dutch flatfish fishery were raised by the European Scientific, Technical and Economic Committee for Fisheries (STECF) and the International Council for the Exploration of the Sea (ICES) and discussed at the meeting of the ICES Working Group on Fishing Technology and Fish Behaviour (WGFTFB) in 2006. These questions led to field strength measurements in situ onboard the commercial beam trawler, and research on the effects of pulse stimulation on cod (*Gadus morhua* L.), and elasmobranch fish. The initial study revealed a potential problem concerning vertebral damage in cod, and suggested only weak responses and no mortality in lesser spotted dogfish or small spotted cat sharks (*Scyliorhinus canicula* L.). caused by the electric stimuli, but due to the strong effect of the measurement protocol on feeding behaviour it was suggested that more experimentation was needed (Van Marlen, *et al.*, 2007). This report gives the results of further experiments on cod.

Experiments on cod were conducted in cooperation with the research station Austevoll of the Institute of Marine Research Bergen, Norway. This station has expertise on many aquacultural studies on cod and salmon and include breeding programmes for cod, salmon and halibut. Against this background, the quality of the experiments, and the availability of healthy fish were issues of importance, which clarifies the choice of this cooperation.

Before the actual start of the project the research methodology was developed with the Norwegian scientist involved and thoroughly discussed on visits prior to the experiments. The final research protocol was sent to the Norwegian research animal board in the summer of 2008 to make sure the research could be conducted under the existing Norwegian animal welfare legislation. The proposed research on cod was expected not to be harmful based on available knowledge. The pilot study on a few lesser spotted dogfish, conducted at the IMARES laboratory, showed there was no visible effect (Van Marlen et al., 2007). Initial approval of the proposed research in August 2008 ensured that there were no limitations to conduct this research at the Norwegian laboratory.

2. Materials and Methods

2.1. Organisation research protocol and procedures

The research was commissioned on 1 September 2008 by the Directorate of Fisheries of the Dutch Ministry of Agriculture, Nature and Food Quality and on 3 and 16 September meetings were held at the Austevoll research station to set up the experiment and to develop all requirements The pulse simulator equipment was equal to the system used in the pilot study on catsharks in 2007 (Van Marlen, et al., 2007) with pulse characteristics equivalent to the nominal menu settings of the commercial full scale system, which represent the average settings of the pulse properties, such as pulse width, amplitude and repetition rate. In practice these settings can be adapted to seasonal conditions and can be varied in a range of +/- 20 % of the nominal condition. In summer-time the amplitude settings are normally reduced by 10 %, while in wintertime these are usually raised by 10 % above the nominal value.

The research was conducted in an outdoor laboratory in four grouped cylindrical tanks, each 3 m in diameter. Each tank was provided with an automated feeding system and a video camera situated 2.5 m above the water surface to monitor fish behaviour. The equipment was controlled from a separated hut, in which also the electric pulse generator was installed. Water was pumped form the adjacent fjord and filtered through a main filter and re-circulated through each tank.

On 19 and 20 September 2008 a pilot study was conducted to test the developed protocol, to get a first impression of the effects, and to check whether additional post-analysis measures would be required. Fish were exposed to the electric stimulus (<5 times) and a very strong effect was observed when fish were in close range of the conductors of the electrodes. In a single case a fish died of vertebral injury (Figure 5) when exposed under nominal pulse settings. As this incident was related to higher conductivity, the pulse amplitude was reduced by 15 % and the current on the electrodes leveled to the value measured during measurements in 2007. Another four fishes were exposed the day after with this reduced stimulus. The responsive behaviour was similar to the behaviour of the fish that were exposed to the nominal exposure. Dissection showed these fishes did not suffer vertebral injury.

After this interim result this reduced pulse amplitude was put in the protocol in a second application that was approved by the Norwegian research animal board on 21 October 2008.

On 13 October 2008 the required number of fish (85 specimen) was taken from the stock of the outer holding net pans and transferred to one of the four indoor tanks. The fish had an average body weight of 1300-1400 gr. with a length ranging from 0.41 to 0.55 m (Table 1). The usual amount of food (consisting of compressed pellets) was offered twice a day. On 23 October the fish were feeding normally and deemed fully acclimatised to the new conditions.

Individual fish were exposed 4 times to the electric stimulus in three different distance ranges from the conductor elements of the electrode pair. As the full-scale system contains 6 consecutive conductors along 4 m long electrodes, the passage of the total length of the full-scale electrodes at a towing speed of 4 knots (i.e. ~2 m/s) would induce 6 exposures in 2 seconds to a stationary fish. Given the result of the pilot study of fish exposed in

close range it was ethically not justifiable to apply more than four exposures. The fish were divided in four groups of 20 specimen each to test the effects in the three distance ranges. A fourth group was kept as control reference and not electrically exposed, These fish were only subdued to the transferring operation in a similar manner to enable discrimination of the effects resulting from transfers from effects caused by the electrical stimuli.

The three exposure categories were:

1. A “far field” distance range of 0.4 m beside a conductor element with very low field strength.
2. A “above field” distance range of 0.2-0.3 m above the centre of a conductor pair with medium to low field strength.
3. A “near field” distance range of 0.1-0.2 m from a single conductor element with the highest field strength.

All exposures were conducted in one sequence on 29 October 2008 over a period of 6 hours in total. The order of execution was “near field”, “above field”, “far field” and finally the transfers of the control group.

Field strength in the described ranges and pulse characteristics were measured the same day according to the methods used on measurements sessions on the electric pulse systems (Van Marlen et al., 2007). Shortly before the exposures, a group of 20 fish were transferred from the main holding tank to a holding tank of 1.4x1.1x0.7 m, which was filled completely with sea water and aerated. From this tank individual fish were transferred to a cage of flexible polyethylene netting (Figure 2) with a triangular profile of 0.6x0.3x0.55 m (length x width x height). The polyethylene netting had a mesh size of 20 mm, wide enough to observe the caged fish by under-water camera. The cage was covered on top with nylon netting (with an opening in the middle to load the fish through) to avoid fish from jumping out of the cage as a reaction to the exposures. With this cage, the fish could be accurately positioned in the required distance range, while the fish was still able to swim freely in the limited area. The sequence of four exposures lasted on average 3 minutes. Each exposure was filmed underwater above the water surface. After the four exposures the cage with the exposed fish was moved through air and the fish immediately released in the corresponding recovery tank, which was within a range of 2 m distance from the exposure tank.

The responsive and behavioural effects of the exposures were observed during and after feeding over a period of 17 days, starting on 31 October 2008 and compared to the feeding behaviour of the fish of the control group. Normal feeding sessions were remotely controlled by computer and set to twice a day (08:00 and 15:00). The food regime was set to 50 % of normal ration to get appetitive behaviour at all feedings. All feeding sessions were filmed.

Table 1 Length and weight of the remaining fish at the end of the experiment

Group	Fish (nr)	Mean Length (m)	StDev	Min (m)	Max (m)	Mean Weight (g)	StDev	Min (g)	Max (g)
Far Field	20	0.48	2.5	0.43	0.52	1383	235	1002	1953
Above field	20	0.49	3.0	0.41	0.53	1460	214	1029	1738
Near Field	16	0.48	2.8	0.44	0.55	1351	229	945	1880
Control	20	0.49	1.9	0.46	0.53	1401	190	1146	1863

2.2. Electric pulse simulator, pulse properties and field strength measurements

The electric stimulus was generated using the Verburg-Holland Ltd. simulator model (Annex 2 Electric pulse simulator), of which the output parameters were similar and thoroughly checked against the full-scale electric beam trawl system used onboard “Lub Senior” UK 153 (Van Marlen, et al., 2007). The simulated electrodes (Figure 1) consisted of a single pair of two electrodes provided by Verburg-Holland Ltd. with equivalent conductors, materials, isolated sections as used in the full-scale system, but with reduced number of conductors (2 pairs instead of 6), reducing the length from 4 m to 0.96 m with an isolated extension of 0.6 m between both conductor of the electrodes. The distance between electrodes was set to the nominal design value of 325 mm. Assuming a towing speed of 4 knots of the commercial pulse trawl system, the electric field around a single pair of conductors will pass a stationary fish in 0.5 s. In order to simulate this dynamic situation the stimulus was set to develop a 1 s burst of pulses of constant amplitude with pulse parameters characterized as “nominal” in the Verburg beam trawl system. The pulse controller was set according the menu settings illustrated in Annex 1 Pulse simulating equipment. The equipment was thoroughly checked by the manufacturer Verburg-Holland Ltd. before it was sent to IMR, Bergen, Austevoll.

2.2.1. Pulse parameters measured

Measurements of the electrical stimulus focused on the main parameters:

- Amplitude in V;
- Pulse width in µs;
- Rise and fall times in µs;
- Repetition rate in Hz;
- Electric field strength in V/m between the electrodes.

2.2.2. Field strength measurements

The pulse characteristics and field strength around the conductors were similar to the outcome of the measurements on the commercial system in the Verburg-Holland facilities and during the first pilot experiment on cat shark in 2007 in the IMARES laboratories (Van Marlen et al., 2007). Field strength was measured on 18 September 2008 in the tank on three axes between the conductor pair of the simulated electrodes (Figure 1 and 3). The results along the B axis are values along the centre of the conductors. The values along the A and C axes were measured 0.1 m off the centre line. Each step represents 0.0345 m. Values were measured with a probe of 25 mm spacing and with probe ends positioned perpendicular to the axes. Peak values were measured opposite the centre of the conductor. The field strength measured on the simulated system are similar to the outcome measured on 25 June 2006 of the commercial system in the Verburg-Holland facilities (Figure 3 and 4).

The field strength corresponding to the “far field”, “above field” and “near field” exposures were respectively 4, 40, and 150 V/m. The field strength result of 192 V/m, measured on 0.1 m (the A axis) opposite the centre of the conductor reduced to 82 V/m when the position is raised to 0.1 m above the bottom. Field strength were also measured inside the cage and no deviations were found with the outcome of the reported values.

2.2.3. Measurement system

The pulse output parameters and field strength in the defined ranges from the conductors of the electrodes were measured shortly before the start of the exposures using a 200 MHz LeCroy WaveSurfer 24XS oscilloscope with

2 differential probes, a high voltage type ADP 305 (SN5069) and a AP031 70V probe for field strength measurements and a CWT Rogowski 60B current probe (0.5 mV/A) to measure the electrode current. Samples of measurement results were stored as JPEG images on hard disc. Electric field strength was measured in the plane of the electrodes with a probe of fixed spacing of 25 mm along three longitudinal rulers with grid units of 36 x 100 mm. These rulers were spaced in the centre between the electrodes, and two 100 mm at either side of the centre ruler. The probe was positioned with the centre on each marker and tips perpendicular to the longitudinal rulers.

2.3. Video observations

The behaviour of the fish during the exposures and the recovery period were observed above the tanks of the four groups of fish using the IMR video camera and recording system. The electric exposures were filmed by using a single IMR ceiling camera repositioned 1.5 m above water level and an IMARES underwater bullet camera in close range of the exposures. All video images were stored on hard disc in MPEG 4 format. An additional WebCam IP camera was positioned above the exposure tank to facilitate remote observations by the commiss-ioner of the research ("Directorate Fish" of the Dutch Ministry for Agriculture, Nature Conservation and Food Quality) and the Dutch manufacturer Verburg-Holland Ltd.

2.4. Environmental conditions

The water level of the tanks during the 14 days recovery period was 1 m and during the electric exposures the water level in that tank was reduced to 0.6 m. Basin water was pumped from the adjacent fjord. Conductivity, temperature and oxygen were measured on a regular basis with IMR equipment (CTD STD204) and during the exposures the salinity was 32.95 mS/cm, temperature 8.2 °C and dissolved oxygen 88 %.

2.5. Analysis of feeding response.

All feeding periods were filmed from the air by a video camera mounted 2.6 m above the centre of the tanks. Feeding automates and video recordings were controlled by computer and additional switching circuitry. The recording started 1 min before feeding and lasted 5 min after end of feeding. The image of the tank was divided in four equal sectors (Figure 6), where the sector down current from the feeding automat was named feeding area. The feeding response was measured as increase in numbers of fish in feeding are from 15 s before feeding to 15 s after feeding. All fish with the snout inside the feeding area where defined as inside.

2.6. Dissection and x-ray analysis

The vertebral column of each fish of the "near field" and "above field" group were carefully dissected, visually examined for pathology, and finally x-rayed and then evaluated for bone fractures on radiographs. Dissected vertebral columns were radiographed by using a portable X-ray apparatus (HI-Ray 100, Eickenmeyer Medizin-technik für Tierärzte e.K., Tuttlingen, Germany) and 0.30x0.40 m film (FUJIFILM IX 100, FUJIFILM Corp., Tokyo, Japan). The film was exposed once for 50 mAs and 68 kV, and developed using a manual developer (Cofar Cemat C56D, Arcore (MI), Italy) with Kodak Professional manual fixer and developer (KODAK S.A., Paris, France). The pictures were digitalised by scanning (Epson Expression 10000 XL, Seiko Epson Corp., Nagano-Ken, Japan).

3. Results

The responses of the “far field” group were minor and only visible occasionally. The fish exposed in the “above field” range showed significant reflexes and muscle contractions, but not as strong as the responses of the “near field” group.

The effects observed on fish exposed in the “near field” range were very strong. During the exposure the fish showed strong reflexes and muscle contractions, which paralysed the fish during the 1 s pulse period. At the moment the pulse train extinguished the fish responded in a disorientated manner with strong and rapid tail actions, expressing a certain shock state, which lasted 3 to 4 s. The four fishes exposed in the earlier pilot study were not held in position in a cage, but could swim through the complete tank volume, or a limited volume of the tank, and swam over a distance of 6 to 9 m. In the present study three fishes exposed in the “near field” range died minutes after the exposures shortly after their release in the recovery tank, a fourth one suffocated after the exposures and was found dead the morning after the exposures. These four fishes were dissected at the research station and showed vertebral injuries (Figure 7). One of the remaining 16 exposed fishes had a black tail, indicating spine injury. In the 14 days observed period another two fishes died, bringing the total to 6. The remaining fish of the “near field” group did not return to normal feeding behaviour. They were not responding to food, except for a few individuals, and most of the time formed a group concentrated near the tank wall outside the feeding zone, and did not show the normal behaviour searching for food. An overview of the effects measured after the exposures, during the observation period and numbers of injured fish is illustrated in Table 2 at the end of this section.

The control group, the “far field” and “above field” group showed a feeding response at the first offering of food after the exposures (about 36h later), but the fish showed relatively low appetite. The “above field” group showed a clear increase in appetite during the recovery period (Figure 8), while the “far field” and the “control” group showed a good feeding response, but the fish did not recover to the feeding response they had before the start of the experiment. Statistical tests (ANOVA) showed a significant difference in feeding response between the treatment groups, and a non-parametric test (Kruskall-Wallis rank sum test) showed a significant difference between the “near field” and control group ($p<0.001$). However, these statistical tests assume that each of the repeated observations (in time) are independent. This assumption of independence is unlikely to be valid given that measurements are made repeatedly in time on the 4 groups of fish in the four tanks. Additionally, the tests do not take into account that there is really only one experimental unit (one group of fish in one fish tank) per treatment. Therefore, the p-values as quoted above are over-optimistic and only indicative.

The radiologic analysis showed that only fish in the “near field” group had pathological signs of bone fracture during dissection and radiological bone fractures. Of the examined fish in this group ($n = 16$), five fish had haemorrhages close to the vertebral column, and of these five, four had radiological bone fractures (Figure 9). This is also including the two fish that died in the recovery period, but not including the 4 injured fish that died directly after the exposures. The vertebral ruptures were found under the third dorsal fin. In total 45 % (9 of 20) of the “near field” group had injuries, while injuries in fish of other groups were not found.

The overview of Table 2 expresses the sorted effects of the electric exposures in the three different exposure ranges.

Table 1 Numbers of fish exposed and results

Exposure category	Nr of fish at the start	Mortality after exposures	Nr of fish in the observation period	Mortality during observation period	Nr of injured fish
Far field	20	0	20	0	Not measured
Above Field	20	0	20	0	0
Near Field	20	4	16	2	5
Control group	20	Not exposed	20	0	Not measured

4. Discussion

The three distance ranges in which the fish were exposed appeared an appropriate way to distinguish the magnitude of effects of the electric stimuli, expressed by the kind of injury, physical reactions without injuries and the distance from where no reactions were observed. Large gradients in stimulus were found in a distance range of 0.4 m from the conductor of the electrodes. The position of cod relative to the conductors is critical in a very narrow range and the physical effects the fish will encounter depend highly on this position, which is also expressed in the measured field strength of which the peak drops 50 V/m over 0.1 m opposite the centre of the conductor (Figure 3).

Fish exposed 0.2 to 0.3 m above the centre of a pair of conductors did not get injured. All fish exposed at a distance range of 0.4 m beside a conductor, representing the fish just outside the fishing range of the trawl, did not react to the exposure and these fish returned to normal feeding behaviour 36 hours after exposure.

The present results clearly demonstrate that cod exposed in close range of the conductors (<0.2 m) can suffer severe injuries, which caused death in 6 out of the total of 20 fish, of which 9 individuals of 16 fish showed spinal injury.

The settings of the commercial system are optimized for catching sole and balanced for the conditions over the year with adjustable pulse amplitude (voltage), pulse width and interval settings in a range of +/- 20 % around the "nominal" values, that were applied in this study. The four fishes exposed in the pilot study to a stimulus with 15 % reduced amplitude did not get vertebral injuries and were tested in the minimum range of the system used in the summertime. This all means that there is an indication that the effects on cod are highest in wintertime.

Vertebral injuries on cod were observed during the commercial trials on board MFV "Lub Senior" UK 153. In the summer of 2007 a sample of 25 cod were taken out of the catch for radiography analysis. It appeared that two fishes had vertebral injuries and another two individuals had vertebral deformations attributed to natural causes (Van Marlen, et al., 2007).

In order to appraise the possible effects on cod in pulse trawl fishery one should distinguish between individuals above and below the minimum landing size (MLS) of 0.35 m. The first question is: how do catches of marketable cod depend on the use of the pulse technique?, the second: what will be the effect on undersized cod?
Two data sources can reveal some insights, the data collected in the comparative fishing trials on FRV "Tridens" in 2004 and 2005 (Van Marlen et al., 2005), and the data of the comparison of the fully equipped beam trawler UK153 with other conventional beam trawlers of 2005 and 2006 (Van Marlen et al., 2006).

An analysis of the FRV "Tridens" LPUE data for cod resulted in higher values for the pulse trawl at a range of pulse field settings, the complete dataset of 67 hauls showed even a significant increase (Annex 2,Table 3). It should be noted, however, that the conventional gear was also towed at the relatively low speed of 5.5 kts, and results may differ at higher towing speeds. Undersized cod was not measured, so there is no comparative data for this category. Secondly the dataset support only 10 hauls with the currently tested nominal pulse setting.

The effects of electric stimuli to smaller juvenile fish (< 0.41 m) were not investigated in this study. A theoretical study on the effects of field strength in relation to fish length (Stewart, 1975) shows that fish reactions to electric fields are length dependent with lower effects for smaller fish. Our results show that the position of the fish relative to the location of highest field strength has a large bearing on the effects of the exposure in the length class tested (i.e. 0.41-0.55 m). Smaller fish need to be positioned more accurately at spots of highest field strength to evoke similar effects. In addition, due to their smaller length, such fish can not maintain a high swimming speed and will likely drop back inside the net sooner than the length classes we tested, thus causing a shorter time of exposure. However, tests on smaller fish were not carried out in our study due to time and budget constraints. Consequently the effects on smaller fish remain unknown and may need further attention.

In the actual situation the fish will most likely react on the presence of the pulses by evading this effect and increasing the distance to the conductors after the first exposure, while in our experiments they received four exposures at 0.1-0.2 m from the conductor as worst case. Secondly the exposure was switched on and off with full electronic speed, while in full-scale the field strength will raise and extinguish more gradually with relative speed of the trawl and cod. This means that the effects under full-scale conditions will probably be less. From experiments with large mesh beam trawls (Van Marlen, 2003) it was sometimes noted that catches of juvenile cod were smaller, which indicated that these fish were escaping through the top panel. This might be a common behaviour causing lower exposure to the pulse field.

The injuries we observed were the result of strong muscle contractions during exposure and were probably aggravated in a shock state of the fish on the moment the pulse extinguished. Disorientated swimming with heavy tail actions indicated such shock behaviour, and lasted 1 to 3 seconds. The disorientated state and the response with rapid swimming may lower the chance of fish escaping from the mouth of the trawl in the towing direction. In all observed cases, including the single case observed in the pilot study, the vertebral injuries were found in the same location under the third dorsal fin (Figure 3, 5 and 6). Fish exposed above the centre of the pair of conductors did show similar but milder muscle contraction during exposure. They did not get injured and responded well to feeding cycles.

From literature and fishing practice it is known that cod stay very close to the bottom in wintertime. The present results indicate that the chances of entering the net close to the sea bed and subsequent vertebral injuries may be higher in this condition. The chances of injuries are further raised by the fact that in wintertime the amplitude settings are above the nominal settings used in other times of the year. Further limitation of the amplitude might be considered.

Vertebral injuries in relation to electric stimuli were reported in many studies. These injuries could be related to characteristics of the electric stimulus, such as amplitude, and pulse shape. In this case the bipolar pattern and the short interval between the positive and negative amplitudes could be an important variable. There is little information about these aspects and if there is, there are many differing results found in different species, depending on experimental conditions and electrical properties. Relevant data on the effects of pulse type are published for electro-fishing on trout (*Salmo gairdneri*). The study of Sharber and Carothers, 1988 showed that

vertebral damage is a common injury related to electro-fishing and occurred in 50 % of the 209 fish examined. Of the tested shapes (quarter-sine waves, exponential pulses, square waves) pulses based on a 60 Hz pulsed output of a 260 DC supply voltage) of quarter sine type had a slightly higher proportion than the other types, but vertebral injuries occurred with all types of pulse shapes and a relation with pulse shape could not be determined.

In most cases exposed cod will be caught in the trawl and the injuries with haemorrhage have a negative effect on the appearance and quality of the fish, which may result in a lower price and/or fish being discarded. But as the fish showed a disorientated behaviour after the exposure and exhibited rapid swimming actions, the fish could theoretically accelerate in the towing direction and escape from the trawl. The question is whether these fish have a chance of survival. In the present study the fish were observed for a relatively short period, in which they hardly responded to feeding cycles. Information on this issue is rare and the outcome dependent on many factors.

The effects of vertebral injuries in rainbow trout (*Onchorhynchus mykiss*) were observed over a long period of 355 days showing that fish with moderate to severe injury (vertebral misalignment and fracture), representing 28 % of the total number exposed, had markedly lower growth and a worse physical condition than fish with no or little vertebral injury (Dalbey and McMahon, 1996).

5. Conclusions and recommendations

The present results clearly demonstrate that cod of the length range tested (0.41-0.55 m) might be affected by pulse stimulation, depending on the position of the fish relative to the conductors of the electrodes.

Cod exposed outside the distance range of 0.4 m from the electrodes, representing fish in the region just outside the trawl, did not react to exposure and these fish returned also to normal feeding behaviour. No effects were found in the “far field” distance range of 0.4 m from a conductor, and only minor effects in the “above field” distance range of 0.2-0.3 m. However, more severe effects occurred in the “near field” distance range of 0.1-0.2 m from a conductor.

Cod exposed in this range can suffer severe vertebral injuries. In the closest range 5 cases showed vertebral injuries (Table 2), while 6 of the 20 exposed fish did not survive the highest exposures. In comparison, no mortality was observed in the other treatment groups. These injuries were the result of strong muscle contractions during exposure. In all observed cases, including the single case observed in the pilot study the vertebral injuries were found in the same location under the third dorsal fin (Figure 3, 5 and 6). Fish exposed above the centre of the pair of conductors did show similar but milder muscle contraction during exposure. They did not get injured and responded well to feeding cycles.

The present study indicates that the pulse amplitude should be reduced by 15 % of the nominal setting to reduce the effects and vertebral injuries. The present pulse settings, however, are optimized for catching sole after many field trials. In wintertime an increase of 15 % is needed to maintain catching efficiency on sole, which further raises the chances of vertebral injuries in cod, as they tend to stay close to the bottom in that season with increased possibility of getting in close range of the electrodes and subsequently being exposed to field strengths of values equivalent to the tested “near field” category or higher (15 % amplitude increase in winter), which can cause injuries found in this study.

Tests on undersized cod were not carried out in our study due to time and budget constraints. Consequently the effects on such fish that may come in contact with the gear, but may not be captured, remain unknown. Being shorter in length the effects will likely be less, but may require further attention.

In most cases cod in the length class tested here will be retained by the relatively small codend mesh size in this fishery (i.e. 80 mm). Spinal injuries and haemorrhages around the vertebrae will have a negative effect on the appearance of the landed fish and its commercial value.

6. References

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Figures and Illustrations

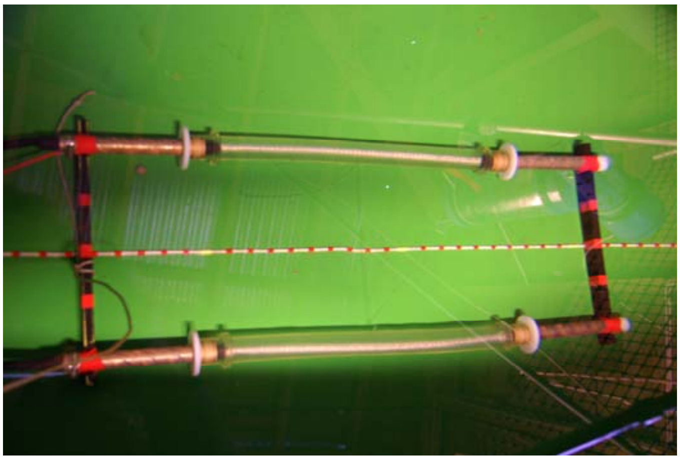


Figure 1. Simulated electrode system with two conductor pairs positioned on the bottom of the test tank with a conductor distance similar to the "nominal" distance of 325 mm. In between the rulers and grid step used to measure field strength between the conductors along three axes alongside the electrodes (Figure 3 and 4).

Figure 2. Overview of all research tanks in the indoor facility of IMR Austevoll, Norway. Tank diameter is 3 m and each tank was provided with a remote feeding unit and a video camera mounted on the ceiling 2. 6 m above the water surface. The normal water level in the tanks is 1 m, during the exposures the water level was reduced to xx m.

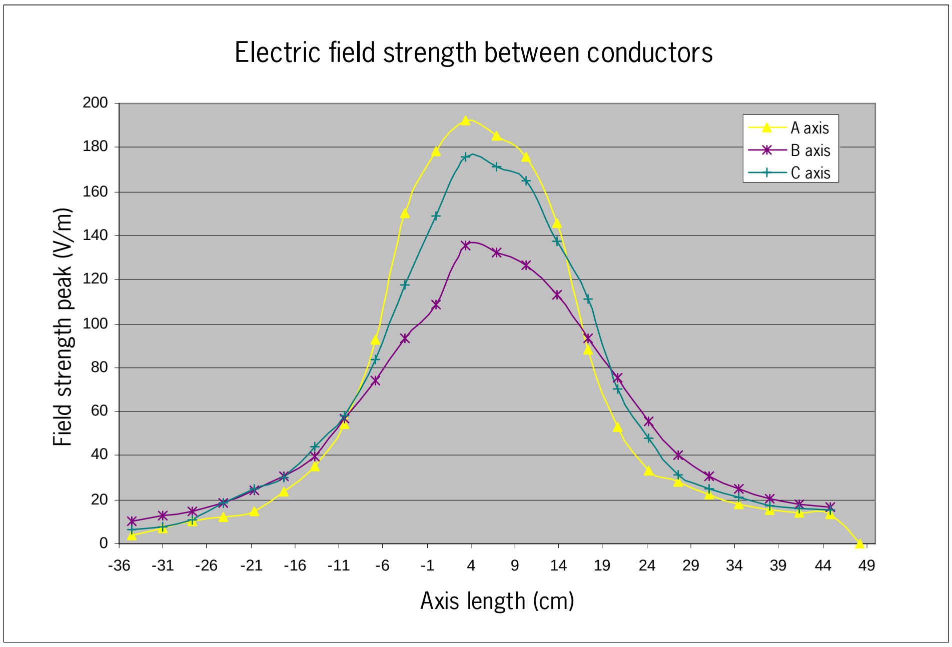


Figure 3. Field strength composition measured on 28 October 2008 on three axes between the conductor pair of the simulated electrodes. The results along the B axis are values along the centre of the conductors. The values along the A and C axes were measured 0.1 m off the centre line. Each step represents 0.0345 m. Values were measured with a probe of 25 mm spacing and with probe ends positioned perpendicular to the axes. Peak values were measured opposite the centre of the conductor

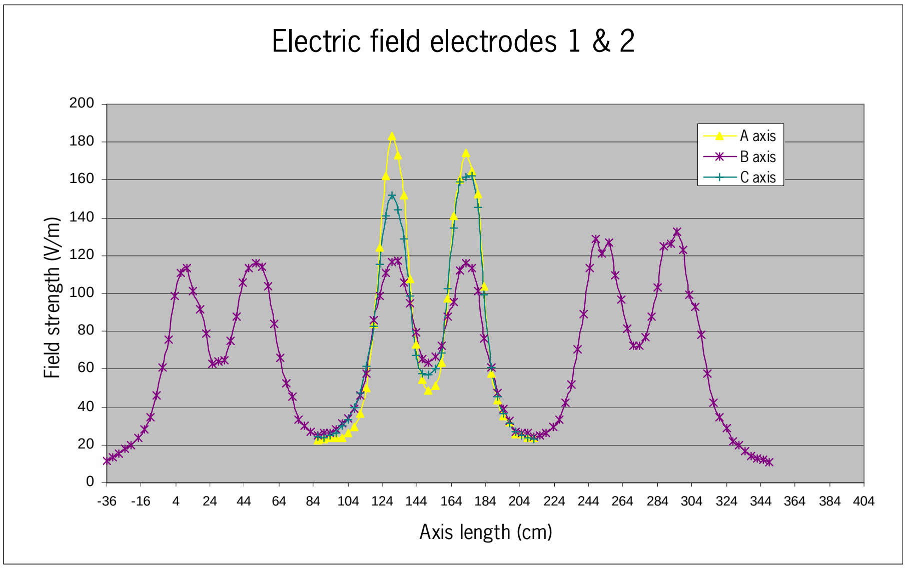


Figure 4. Field strength composition measured on 25 June 2006 on the commercial electrodes on three axes between the electrodes conductors 3 and 4. Values of the B axis were measured along the centre of the conductors. The values along the A and C axes were measured 0.1 m off the centre line. Each step represents 36 mm. Values were measured with a probe of 25 mm spacing and with probe ends positioned perpendicular to the axes. The measured pattern represents the 6 conductors with peaks opposite each centre and the drops as a result of the 0.2 and 0.6 m isolated extensions.

Figure 5. Cod exposed to "nominal" pulse exposure in the pilot study of 18 September 2008 showing vertebral injury

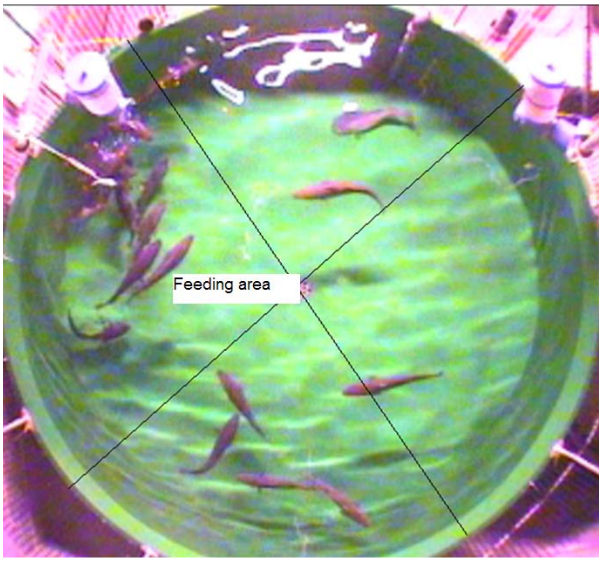


Figure 6. Fish tank used in the recovery period, and the sectors in the tank used to quantify feeding response.

Figure 7. The four dissected fish, that died shortly after the exposures with vertebral injury all in the same area of the vertebrae.

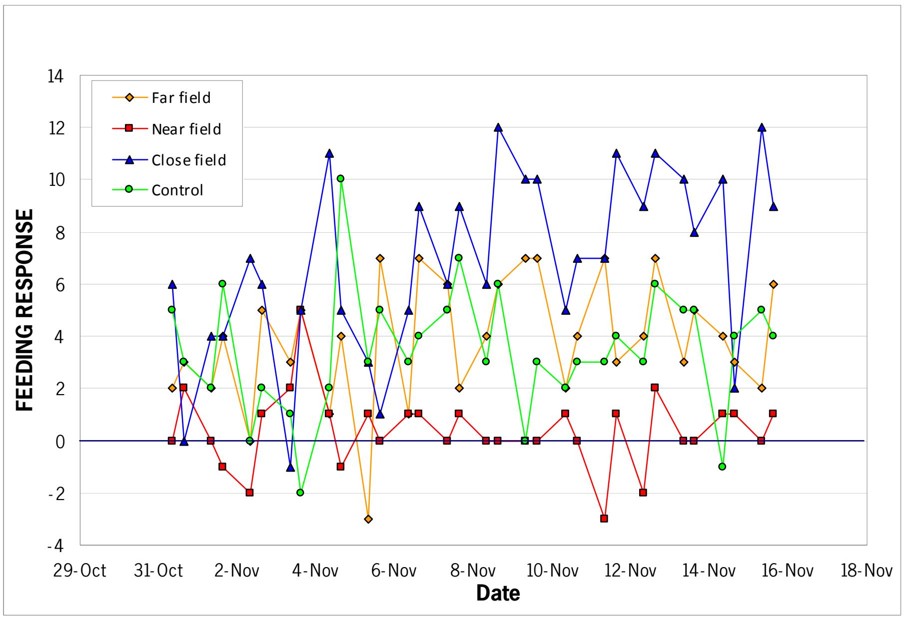
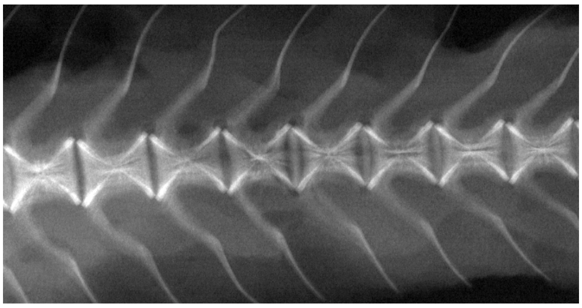
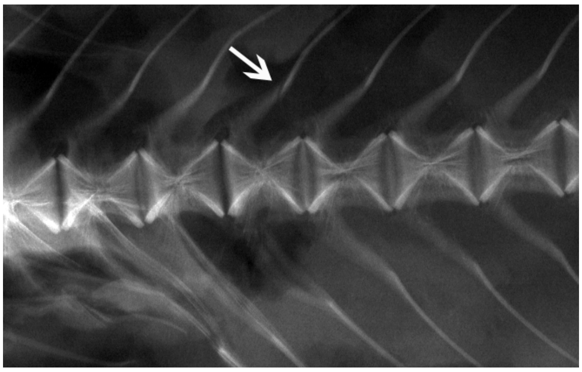


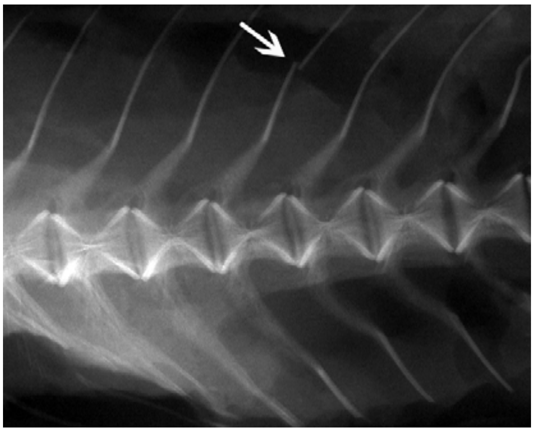
Figure 8. Increase in numbers of fish in the feeding area from 15 s before feeding to 15 s after feeding in a defined feeding area (Figure 6). Points above the blue line indicate a positive response.The result shows a significant and very clear difference in the response of the "near field" group and the groups of fish exposed at longer distances from the conductors.



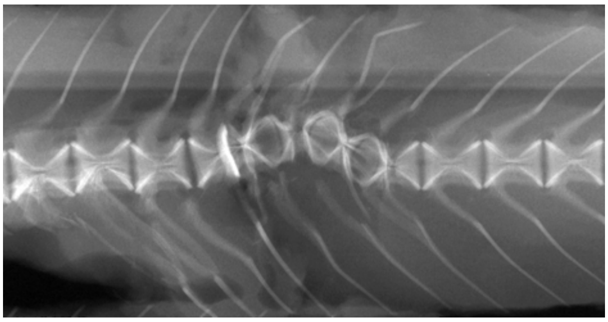
a) An individual with haemorrhage and no fractures.



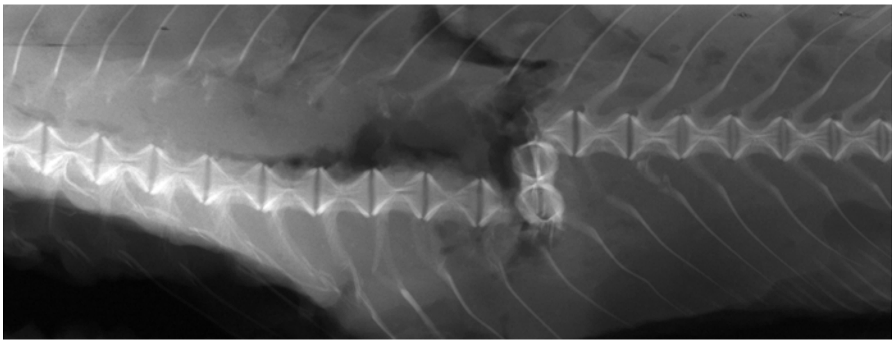
b) An individual with haemorrhage and fracture in neural arch.



c) An individual with a haemorrhage and fracture in neural arch.



d) An individual with haemorrhage and ruptured spinal column.



e) An individual with a haemorrhage and ruptured vertebral column.

Figure 9 (a-e). Lateral radiographs of the 5 injured individuals exposed in the "near field" range of the conductors All five fish had haemorrhages and some had severe fractures that involved both a rupture of the vertebral column and fractures between the haemal arches.

Annex 1 Pulse simulating equipment

The pulse generating equipment consists of the following main parts:

An adjustable power supply (A);
Pulse generator (B);
Output inductance (C);
Electrodes (D);
Oscilloscope (E).

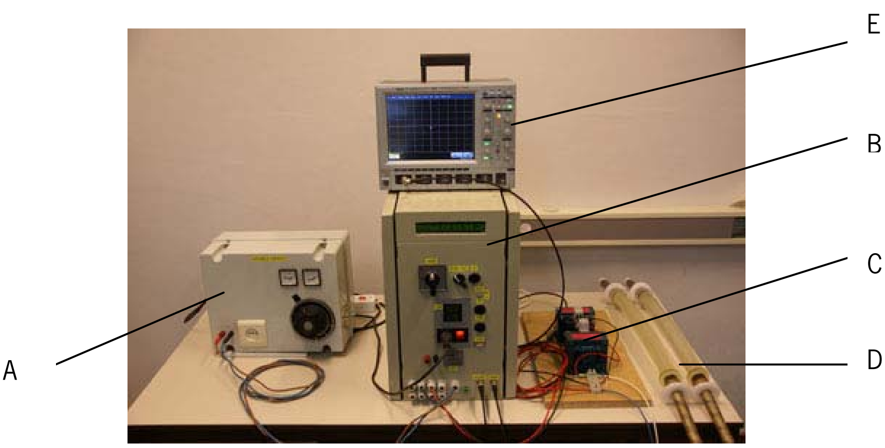
Adjustable power supply (A)
Power supply output provides a DC intermitting voltage of 0-200 V. For the experiment the voltage was set to 100 V.

Pulse generator (B)
The pulse generator consists of a microprocessor and a pair of IGBT's. With the controller the main parameters of the stimulus, amplitude, pulse shape, pulse width etc. are programmed.

Menu settings

Pulsevorm	(Pulse shape)	P1;
Length T3	(Burst length)	1.0 s;
Period T2	(Pulse period)	nominal;
Pulse T1	(Pulse width)	nominal;
Powervorm	(Power shape)	P0;
Cont/single	(pulse out)	s single;
Number T4		Overruled by T3;
Aantal	(number)	1
Powerinst.	(power setting)	Amplitude 88 %

Equipment layout



Annex 2 Comparison of cod catches of pulse trawl against the conventional trawl

Table 3 LPUE of pulse beam trawl (PULSE) and conventional tickler chain beam trawl (CONV) for various pulse field settings, with mean, stdev and p-value for category cod > MLS. Boldface values are significant (p <= 0.05).

Gear test	Pulse setting	No of hauls	kg/hour					
			mean			stdev		p-value
			PULSE	CONV	PULSE/CONV	PULSE	CONV	
1	b+10	14	0.99	0.62	159.7%	1.59	0.94	0.687
2	b+20	12	2.55	0.81	314.8%	2.33	1.12	0.062
3	b-10	2	3.32	0.00		1.7	0	0.104
4	h+10	16	0.84	0.71	118.3%	1.13	1	0.620
5	h+20	10	0.64	0.51	125.5%	0.94	0.44	0.864
6	h-10	2	0.60	0.00				
7	h-20	1	0.43	0.00				
8	nominal	10	2.24	0.67	334.3%	1.96	0.8	0.246
9	total	67	**1.39**	**0.61**	**227.9%**	**1.71**	**0.86**	**0.017**

The second dataset, based on the auction data of the comparison in 2006, gave different results for cod landings using the generalized linear model of Van Marlen et al., 2006 with the pulse trawl catching fewer marketable cod (Table 4). Note that towing speeds differed, the pulse trawl having 5.5 kts and the conventional 6.5 kts on average.

Table 4 Comparison of landings of cod based on auction data between a beam trawler fishing with two pulse trawls and two conventional beam trawlers fishing in the same area and time (based on Van Marlen et al., 2006).

Effect	Length range (m)	Dependent	GEAR	LowerCL	LSMean	UpperCL	Probt	Significance
GEAR	> 0.88	cat1_CPUE	Conv	0.0607	0.4371	0.8135	0.2012	n.s.
			Puls	-0.1815	0.1949	0.5713		
	0.72 – 0.88	cat2_CPUE	Conv	0.2036	0.7612	1.3188	0.1170	n.s.
			Puls	-0.1069	0.3251	0.7570		
	0.55 – 0.72	cat3_CPUE	Conv	-0.1199	0.9058	1.9316	0.4074	n.s.
			Puls	-0.3316	0.5059	1.3434		
	0.46 – 0.55	cat4_CPUE	Conv	-0.0037	0.5161	1.0359	0.2256	n.s.
			Puls	-0.4965	0.1746	0.8457		
	0.35 – 0.46	cat5_CPUE	Conv	0.2139	0.2348	0.2557	0.0392	s.
			Puls	0.1334	0.1752	0.2170		
		cat6_CPUE	Conv	n/a	0.1281	n/a	n/a	n/a
			Puls	n/a	n/a	n/a		
	All	tot_CPUE	Conv	0.7664	2.2559	3.7455	0.1954	n.s.
			Puls	-0.4117	1.0778	2.5674		

Apparently the outcome of the analysis of these two datasets contradict, which means that a firm conclusion about differences in catch of marketable cod by the pulse trawl compared to the conventional beam trawl can not be made.

Table 5 Discard rates of cod, results analysis of variance. Legend: +++: P<0.01, n.s.: 0.10 < P.

Species	Unit W/N	Obs (hauls)	Comparison of means			R^2
			Average Pulse in kg/hr	Average Conventional #/hr	Difference significance	
Cod (trip 1 t/m5) <35 cm	N	148	5.854	4.602	n.s.	0.36
	W	148	0.630	0.442	n.s.	0.24

Discards were also sampled during these trips. The analysis of these data indicated that but more undersized cod were caught in the pulse trawl, but the result was not significant (Table 5).

The fate of marketable cod will differ from undersized cod, as marketable cod will be retained by the meshes of the codend (80 mm) and not survive. Our tests indicate that some, but not all, of these fish might get damaged when they enter the net close to the sea bed, which will likely deteriorate their market value. Undersized cod might have a chance of escapement and survival, depending on their length.

Referees and Authors

Rapport C098/08
Project Number: 4391300702

This report has been professionally prepared by Wageningen IMARES. The scientific validity of this report has been internally tested and verified by another researcher and evaluated by the Scientific Team at Wageningen IMARES.

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Quantitative biologist

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Date: 05 November 2009

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Date: 05 November 2009

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