The effect of pulse stimulation on biota – Research in relation to ICES advice – Progress report with preliminary results.

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Report number C098/07



Institute for Marine Resources and Ecosystem Studies

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Publication Date: 16.10.2007

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# Assignment

The work has been carried out in the context of EU Project "Development of fishing Gears with Reduced Effects on the Environment" (DEGREE, SSP8-CT-2004-022576) in close consultation with the co-financing authority Directorate of Fisheries of the Ministry of Agriculture, Nature and Food Quality of the Netherlands, and the private company Verburg-Holland Ltd. of Colijnsplaat, the Netherlands.

# **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 12<sup>th</sup> 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 31 May and 5 October 2007.

#### These activities involved:

- 1. Measurements on the detailed stimulus applied in the pulse trawling system developed by the company Verburg-Holland Ltd., i.e. the amplitude, pulse width, rise and fall times, repetition rate and field strength along the electrodes. These measurements were done onboard of the commercial fishing vessel MFV "Lub Senior" (UK153), and in tank facilities of the manufacturer of the pulse beam trawl.
- 2. Simulation of this stimulus in the recirculated aquaculture system available at IMARES
- 3. Development of a protocol for keeping small-spotted catsharks alive and well, including dietary requirements.
- 4. The exposure of catsharks to a simulated pulse under laboratory conditions and observation of behaviour, including foraging, and monitoring mortality
- 5. Investigation of possible spinal damage of cod caught by a commercial vessel using pulse beam trawls by X-ray photography.

The electric pulse characteristics were measured onboard MFV "Lub Senior" UK153 at sea. Shortly after these measurements the complete system including trawl winch became available for measurements in the Verburg-Holland Ltd. reference basin with fixed salinity (specific conductance). Based on this outcome a pulse simulator system was developed to be used in the experiments on fish in tanks of IMARES. This stimulus of this system was proven to be electrically equivalent. The highest electric field strength measured in this reference basin was 176 v/m, with a specific conductance of 57.1 mS/cm.

The analysis of X-ray scans revealed that 2 out of 25 fish had a dislocated spine. In addition 6 animals out of the group of 25 showed deformations which can be attributed to natural causes. Although the sample size is small any effect from the pulse stimulation can not be ruled out, but it appears to be low in percentage, and still needs to be compared to fish caught with the conventional system. Therefore any definite conclusions can not be drawn at this stage.

Guidelines for husbandry and assessment of responses in behaviour, including foraging, to exposure of the electric field were developed. A first experiment involved two single fish. An individual tagged catshark was exposed to the electric field and its behaviour was compared to that of a control fish. No response in behaviour in this fish could be observed, and mortality did not occur. Based on this preliminary trial a protocol was drafted to assess effects in behaviour of groups of sharks to the electric field. A second experiment was done later on two groups of catsharks, one group exposed to an electrical stimulus and the other not, thus serving as a control group. From this it was found that transferring these fish from a holding tank to a separate tank in which the stimulus can be applied does affect feeding behaviour. This finding will be used to improve the experimental design. In addition no mortality was seen in the two groups, indicating that the stimulus did not have a noticeable immediate effect.

It should be stressed out the work is still continuing and the results obtained so far are of a preliminary nature. By actually carrying out these experiments we learn more about the difficulties of keeping fish alive in good condition and inferring from their behaviour and mortality the effects of the electrical stimulation on these species.

# Introduction

#### Background and research carried out so far

There is growing concern about the impact of fishing on marine eco-systems, and particularly on the benthic fauna. Beam trawls are intensively used in the North Sea fisheries of the Netherlands, Belgium, Germany, and the United Kingdom. These gears are fished with relatively heavy groundgear and relatively high towing speed (e.g. 6.5 to 7.0 kts) and are causing substantial mortality and possible changes in the species composition of invertebrates (Anon., 1988, 1995; Jennings and Kaiser, 1998; Lindeboom and De Groot, 1998; Kaiser and De Groot, 2000; Fonteyne and Polet; 2002; Piet *et al.*, 2000). A study revealed that the penetration depth of beam trawls varies between 1 and 8 cm, depending on the type of gear and substrate (Paschen *et al.*, 2000).

Electrical stimuli evoke reactions in fish ranging from a startling response to narcosis (McBary, 1956). In freshwater direct current can be used to attract fish by forced swimming (anodic attraction). Research on electrical or pulse stimulation in beam trawling was carried out extensively from 1970, in the Netherlands (De Groot and Boonstra, 1970, 1974; Agricola, 1985; Van Marlen, 2000), Belgium (Vanden Broucke, 1973), Germany (Horn, 1976) and the United Kingdom (Horton, 1984). In seawater a pulsing electric field can be utilised to chase flatfish, in particular sole (Solea vulgaris L.) out of the sea bed. An array of electrodes can be used to replace tickler chains in beam trawls (De Groot and Boonstra, 1970, 1974). The possibility of size selection was raised, as longer fish were expected to react more strongly (Stewart, 1975), although not clearly confirmed later by experiments (Stewart, 1978, Agricola, 1985). The primary motive at that time was to save fuel by decreasing gear drag, and the potential for using this technique for catching shrimps and flatfish was shown. In spite of the development of various prototypes introduction in commercial practice never happened (Van Marlen, 1997). At present a main objective is to reduce the impact of ground gear on the sea bed. Any successful new stimulation technique should offer adequate catch levels on target species, sound economics, a decrease in by-catch levels, similar chances of survival for escaping and discarded animals, and no effect on the reproductive capabilities of the species affected.

Wageningen IMARES (former RIVO) became again involved in 1998 in a research and development programme started by the Ministry of Agriculture, Nature and Fisheries. A pulse trawl with a beam length of 7 m produced by a private company was extensively tested in that year. These trials resulted in sole catches of the same magnitude and lower catches of plaice (*Pleuronectes platessa* L.) and benthos. These promising results led to follow-up experiments in 1999 with a modified gear. The first objective was to improve the catches of plaice, appraise the effect of towing speed, compare the warp loads of both gears, and appraise the effect of the electrical stimulation on short-term fish survival. The second objective was to further improve the catching performance of the net attached to the beam of the pulse trawl, and to collect more data on short-term survival, also of benthic animals (Van Marlen, *et al.*, 1999; Van Marlen, *et al.*, 2000; Van Marlen, *et al.*, 2001a, 2001b).

Beam trawling for flatfish is an efficient fishing method, but it requires a high level of energy input, due to the high gear drag and towing speeds, and affects benthic fauna (De Groot and Lindeboom, 1998). This has led to research on alternatives, such as electrical stimulation, initially aimed at reducing gear drag and fuel consumption (Agricola, 1985). Prototype gears were developed for shrimps and flatfish fisheries, but until the present day a commercial application did not emerge (Van Marlen and De Haan, 1988; Van Marlen, *et al.*, 1997). Fishing with electricity was banned in the European Union (EU) in 1988. The reason for this was fear of increasing catch efficiency in a time when the discrepancy between the state of the resources and the ever increasing fishing

effort became problematic. In the late 1990s the development of beam trawling with electrical stimulation was continued, but now the focus was on reducing adverse ecosystem effects (Van Marlen, *et al.*, 2001a).

Wageningen IMARES became involved in an existing trilateral cooperation between a private company (Verburg-Holland Ltd.), the Dutch Fishermen's Federation and the Ministry of Agriculture, Nature and Food Quality in 1998. A series of trials were conducted onboard FRV "Tridens" on a 7 m prototype electrified beam trawl, called 'pulse' trawl, resulting in sole (*Solea vulgaris* L.) catches matching those of conventional tickler chain beam trawls, plaice catches being reduced by about 50%, and benthos catches reduced by 40%. These results stimulated further work. Extended trials were carried out in October-November 1999 (Van Marlen, *et al.*, 1999; Van Marlen, *et al.*, 2000).

A study on differences between a conventional 7 m tickler chain gear and the 7 m prototype electrical gear in direct mortality of invertebrates living on and in the sea bed was conducted in June 2000 onboard FRV "Tridens" and RV "Zirfaea". Benthos samples were taken from the Oyster grounds prior to fishing, and from trawl tracks caused by the two gear types. The direct mortality calculated from densities in these samples was lower for an assembly of 15 taxa for the pulse trawl, indicating the potential of electrical fishing to reduce effects on benthic communities (Van Marlen, *et al.*, 2001).

After these experiments it was decided to develop a prototype for 12 m beam length, being the most common value in the Dutch fleet. Technical trials with the new prototype were carried out in November-December 2001 onboard FRV "Tridens", and continued in 2002 and 2003, resulting in catch rates for sole and plaice equalling those of conventional 12 m gear.

Recently the bycatch and discarding of undersized fish, particularly plaice (*Pleuronectes platessa* L.) gained attention. Comparative studies were undertaken in 2005 on FRV "Tridens" on the differences in catches and on differences in survival of undersized sole and plaice between a 12 m pulse beam trawl and a conventional 12 m tickler chain beam trawl (Van Marlen *et al.*, 2005a, b). A higher survival rate for plaice, but not for sole, was found for the pulse trawl, while the level of blood parameters (glucose, free fatty acids, cortisol, and lactate) and the changes over time in blood samples taken from both species showed no significant differences between both stimulation techniques.

In the fall of 2004 it was concluded that the 12 m prototype was technically ready for a series of long-term trials on a commercial fishing vessel. The Motor Fishing Vessel (MFV) UK153 "Lub Senior" was outfitted with a complete system of two pulse trawls and cable winches. A series of experiments was carried out on the UK 153 in the period between October 2005 and March 2006 and compared to the performance of similar beam trawlers fishing with the conventional gear type in the same period, and on the same fishing grounds in the North Sea, on the Dutch Continental Shelf. The MFV UK153 was outfitted with a complete system of two pulse trawls and winches with feeding cables. Nine trips in total were undertaken. Five trips were used to make actual comparisons with a second vessel (Van Marlen *et al.*, 2006). The main findings were that landings of plaice and sole were significantly lower, but there was no significant difference in the catch rates of undersized (discard) plaice between the pulse trawl and the conventional trawl. In the pulse trawl, the catch rates of undersized (discard) sole were significantly lower than in the conventional beam trawl. The catch rates of benthic fauna (nrs/hr of *Astropecten irregularis*, *Asterias rubens*, and *Liocarcinus holsatus*) were significantly lower in the pulse trawl. Also, as found before, there were indications that undersized plaice is damaged to a lesser degree and have better survival chances in the pulse trawl.

#### Questions raised by the European Commission addressed to ICES

The European Commission has requested ICES to evaluate the possible effect of the use of pulse-trawl electrical fishing gear to target plaice and sole in beam-trawl fisheries:

- a. What change in fishing mortality could be expected following the adoption of such gear in the commercial fishery, assuming unchanged effort measured in kW-days at sea?
- b. What effect would such a widespread introduction have in terms of (i) the mixture of species caught; (ii) the size of fish caught?
- c. What, if any, effects would such introduction have on non-target species in the marine ecosystems where this gear was deployed?

#### ICES conclusion and recommendation on additional data needs

The following ICES Conclusion was articulated:

"The available information shows that the pulse trawl gear could cause a reduction in catch rate (kg/hr) of undersized sole, compared to standard beam trawls. Catch rates of sole above the minimum landing size from research vessel trials were higher but the commercial feasibility study suggested lower catch rates. Plaice catch rates decreased for all size classes. No firm conclusions could be drawn for dab, turbot, cod and whiting but there was a tendency for lower catch rates.

The gear seems to reduce catches of benthic invertebrates and lower trawl path mortality of some in-fauna species.

Because of the lighter gear and the lower towing speed, there is a considerable reduction in fuel consumption and the swept area per hour is lower.

There are indications that the gear could inflict increased mortality on target and non-target species that contact the gear but are not retained.

The pulse trawl gear has some preferable properties compared to the standard beam trawl with tickler chains but the potential for inflicting an increased unaccounted mortality on target and non-target species requires additional experiments before final conclusions can be drawn on the likely overall ecosystem effects of this gear."

The recommendations of ICES are given below:

"Further tank experiments are needed to determine whether injury is being caused to fish escaping from the pulse trawl gear. The experiments need to be conducted on a range of target and non-target fish species that are typically encountered by the beam trawl gear and with different length classes. In these trials it should be ensured that the exposure matches the situation *in situ* during a passage of the pulse beam trawl. Fish should be subjected to both external and internal examination after exposure.

If the pulse trawl were to be introduced into the commercial fishery, there would be a need to closely monitor the fishery with a focus on the technological development and bycatch properties."

In response to this advice further research was suggested, in consultation with the Directorate of Fisheries of the Ministry of Agriculture, Nature and Food Quality (LNV) it was decided to concentrate on the topics below:

- Study into spinal damage in cod (*Gadus morhua* L.)
- Research into the effects of electrical stimuli on elasmobranch fish.
- Further study into the effects on benthic invertebrates that are subject to the electric field generated by the electrodes in the gear.

The following objectives were chosen for this preliminary study:

- To assess potential spinal damage of cod landed by the commercial fishing vessel MFV "Lub Senior" (UK153) fishing with pulse beam trawls and use X-ray scanning and photography.
- To measure the characteristics of the stimulus used in practice in pulse trawling, *i.e.* amplitude, pulse width, rise and fall times and repetition rate and field strength along the electrodes.
- To simulate this stimulus in the recirculated seawater tanks at IMARES
- To develop the methodology and carry out a first experiment to investigate the effects of exposure of small-spotted catshark (*Scyliorhinus canicula*) to a simulated pulse stimulus under laboratory conditions and monitor behaviour, including foraging, and mortality.
- To define behavioural indicators for sound husbandry of small-spotted catsharks in the IMARES' recirculated tanks, including dietary requirements

In the report we described results obtained in the period 31 May- 12 October 2007. In this period only experiments with small-spotted catsharks and landed cod were carried out. For reasons of logistics it was not possible to keep both live catsharks and thornback rays simultaneously in the recirculation aquaculture system at IMARES. The reported study started with catsharks.

# Materials and Methods

Measurements on the electric system stimulus in a laboratory and *in situ* onboard MFV "Lub Senior" (UK153).

#### Objective

The objective of these measurements were:

• To make sure that the electrical stimulus of pulse simulator to be used in the tank experiments on fish and other biota is a good match of the stimulus of the UK153 pulse trawl system at sea.

#### Systems measured

Three systems were measured, *i.e.* the complete commercial system operated at sea onboard of MFV "Lub Senior" (UK153) consisting of two pulse beam trawls with feeding cables and winches both measured at sea and

in the test basin of Verburg-Holland Ltd.; a prototype pulse trawl system measured in a test basin at Verburg, and a single pair electrode system to be used for tank experiments on fish behaviour to simulate the actual *in situ* stimulus was measured at the test basin of Verburg and checked during the experiments in the laboratory tanks of IMARES. The reference measurements took place between 31 May and 11 September 2007. All three systems were tested at the same reference conditions in the Verburg basin (Table 1).

Table 1: Systems and test sites

System tested	System features Location:	at sea	at Verburg	at IMARES
Single pulse	Prototype pulse beam trawl (not fully	-	Х	-
trawl	equivalent to the UK153 system, with an			
	earlier version of electrodes). This systen	ı		
	consists of 30 electrodes and six conduc	tors		
	per electrode with isolators in between.			
UK153-	Complete onboard system with two pulse	Х	Х	-
system	beam trawls, feeding cables and winches	. This		
	system consists of 30 electrodes and six			
	conductors per electrode with isolators ir	1		
	between.			
Pulse	Single pair of two electrodes with reduce	d -	Х	X
simulator	length (960 mm) and two conductor segn	nents		
	on both ends, and an isolator in between.	The		
	dimensions and materials of these electro	odes		
	were fully equivalent with those of the UK	153		
	system.			

#### Pulse parameters measured

Measurements of the electrical stimulus focused on the main parameters:

- Amplitude;
- Pulse width;
- Rise and fall times;
- Repetition rate;
- Electric field strength measurements between the electrodes.

#### Indoor basin at Verburg-Holland Ltd.

The dimensions of the basin were  $12.5 \times 5 \times 0.35$  m. A sand layer of 8-12 cm was present on the bottom. The basin was filled with seawater, which was neither refreshed nor filtered during the measurements. Therefore, salinity increased in time. The water level was kept constant by adding seawater stored in another basin, filled manually with water from the Eastern Scheldt (Oosterschelde).

#### Data measured and equipment used

The measuring equipment consisted of a 200 MHz LeCroy WaveSurfer 24XS oscilloscope with a differential high voltage probe type ADP 305 (SN5069) and a CWT Rogowski 60B current probe (0.5 mV/A). Samples of measurement results (screen images) were stored as JPEG images on hard disc. Environmental data, influencing the outcome, such as salinity and temperature were measured during all experiments with a Hydrolab data sonde, type DS5A serial nr. 38910 (Hydrolab SN44909), and were conducted shortly before the start of the measurements and fish behaviour experiments. During the measurements the spacing of the electrodes was set to the design value of 325 mm, a basic setting for all systems measured. Electric field strength was measured in the plane of the electrodes with a probe of fixed spacing of 25 mm along three longitudinal rules with markers of 36 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 perpendicular to all rulers.

#### Gear components of the pulse beam trawl

The gear components of the pulse beam trawl consist of 30 parallel electrodes, configured in five transformer groups with six electrodes per group. Each electrode consists of six conductors, paired in serial with adjacent electrodes on the secondary side of each transformer (Table 1).

#### Pulse simulator

A pulse simulator to be used in tank experiments in the laboratory on marine biota was made to produce a electrically fully equivalent stimulus to the UK153 system. The simulator consisted of a single pair of two electrodes made with the same dimensions and materials as the UK153 system with reduced length (960 mm) and two conductor segments on both ends, and an isolator in between (Table 1).

Due to the limited dimensions of the tanks at IMARES ( $2 \times 2 \text{ m}$ ), the number of conductors was reduced to two for each electrode, The distance between the electrodes was set to the design spacing of 325 mm. This electrode system was positioned at the centre of the IMARES tank. All basic components were made available by Verburg-Holland Ltd..

Assuming a 4 knot towing speed of the commercial pulse trawl system in the field, the electric field will pass a stationary fish in 1 s. In order to simulate this dynamic situation the electrical simulated 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. Field strengths and main parameters of the pulse were measured in advance of the tank experiments and after each session in the position of the animal on activation of the stimulus.

#### Confidentiality

Details of the pulse trawl system developed by Verburg-Holland Ltd. and in particular the characteristics of the stimulus are confidential and therefore not revealed in this report. Technical details are given in a confidential detailed report. This report is only available to technical experts of Wageningen IMARES and Verburg-Holland Ltd. and members of the ICES Expert Group who confirmed confidentiality.

#### Study into spinal damage in cod (*Gadus morhua* L.)

Cod were caught and gutted onboard MFV "Lub Senior" UK153 in the week of 30 July - 3 August 2007, and transported to the laboratory. The fish were then wrapped in plastic foil and frozen by placing them gently stretched out on the floor of a freezer room (-25 °C) the same day. One day before analysis by X-ray the fish were placed in our laboratory at room temperature for thawing.

At the chair group Experimental Zoology of the Wageningen University the fish were X-rayed in order to investigate spinal dislocations or deformities. The X-raying setup consisted of a Philips Optimus M 200 X-ray apparatus and a 9/5 inch image intensifier in combination with a Plumbicon Video chain. We made lateral X-ray video images of the fish to screen the batch. X-ray pictures were also taken of animals in which we observed spinal column abnormalities. The X-ray photographs were made from both the lateral- and dorsoventral side. The advantage of X-ray pictures is, that they improve the resolution of the images compared to video images and next to it we had hard copy of the suspected spinal columns for closer investigation. The distance between the X-ray tube focus and the fish was 85 cm. The small focus of the X-ray tube (0.6 mm²) was chosen to improve the resolution of the X-ray photographs and KODAK MIN-R film was used, which yielded superb resolution, detail, and contrast.

Development of methodology of experimentation with small-spotted catshark (*Scyliorhinus canicula*)

#### Collection, transport, and keeping fish at IMARES

Small-spotted catshark were caught by the fisheries research vessel (FRV) "Tridens" of the Ministry of Agriculture, Nature and Food Quality in February 2007. The fishing gear used was a otter trawl (G.O.V. gear) in the South Western part of the North Sea.

As soon as possible after arrival of FRV "Tridens" in the harbour of Scheveningen, the fish were transported to IMARES in IJmuiden. The trip lasted approximately 1 hour. For transportation the fish were kept in two tanks (width 0.71, length 1.08 m, height 0.70 m, water volume 385 l). During transportation the water was oxygenated by using a bottle with compressed oxygen in combination with a diffuser that was present on the bottom of each tank. Upon arrival the fish were placed in three tanks ( $2 \times 2 \times 0.5 \text{ m}$ ). Coarse sand was present on the bottom of the tanks to simulate the natural habitat. The water was purified by using our filter systems and re-circulated. The water temperature was approx 17 °C and the oxygen content was 70%. On 26 July the fish were transferred from the third tank to the other tanks to reduce the number of tanks from three to two.

#### Tagging of small-spotted catshark

Some sort of marker is needed to distinguish treated fish from the control group in a tank. Three days before of the experiment on individual catsharks, one fish from each holding tank was labelled by insertion of a floy tag at the dorsal fin. We decided not to anaesthetize the two fish, as a protocol for anaesthesia is not available for this species. The use of an sub-optimal procedure for anaesthesia may slow down breathing, which may ultimately result in death. In addition, it is known that the application of anaesthesia and recovery from this may cause stress. In order to facilitate labelling the fish was placed on chopping board and the eyes were covered by a towel.

#### Indicators to monitor welfare during husbandry of small spotted catsharks

The Harderwijk Marine Mammal Park in the Netherlands gave us advice concerning husbandry of catsharks based on their profound knowledge and experience to ensure optimal welfare of the sharks in our laboratory facilities. This made it possible to draft a list of indicators to monitor behaviour of the species with regard to swimming, resting, feeding behaviour, and study deviations form normal behaviour. In addition, the Harderwijk Marine Mammal Park provided IMARES with advice on dietary requirements. Such a list of indicators depends on the existing facilities to keep the animals and can therefore not regarded as generally applicable. It will be improved as new knowledge will emerge from the currently planned experiments. In this sense one can regard this as a learning process.

#### Video registration and light conditions

The room in which the tanks were placed was kept under the following light regime: during the day: from 0600 to 22.00 hrs (normal TL-lights on), and at night: from 22.00 to 06.00 hrs (weak illumination). A very sensitive low-light video camera (SIMRAD OE 1323) camera was placed above the experimental tank at IMARES, and two low sensitive (0.5 lux) cameras above the two holding tanks. The light level above the experimental tank of IMARES was measured with a Lutron LX-107 Light meter. The light level (at the time of the experiments) in the holding tank for the control group was 60-70 lux (centre at water surface). The light level holding tank for the exposed group was 80-100 lux (centre at water surface). Prior to the experiments the light levels were slightly lower and were increased to optimise the conditions for video observations

Video signals were recorded on Sony GV-D 1000 monitor recording systems and stored on mini DV format. The recordings were scheduled according to the experimental design and were recorded continuously one hr before and one hr after experimentation, and then reduced to a schedule of five minutes per hour. The resolution of video recordings was found to be satisfactory to assess behaviour using the artificial illumination.

Experimental design for assessing the effect of electrical stimuli on the behaviour of small-spotted catshark

A preliminary experiment was carried out with two tagged catsharks to develop a proper experimental design for exposure of individual fish to the pulsed electric field. The control fish and the fish that was exposed to the pulsed electric field in a separate tank (the experimental tank) were caught by a hand net from two separate holding tanks.

The tagged control fish, was transferred to the experimental tank. Here, the fish was monitored for approximately 30 min. after which the fish was transferred to the tank from which it came from. Subsequently, the other tagged catshark was transferred to the experimental tank and after 15 min. exposed to the pulsed electric field for 1 s. Then 15 min later the fish was transferred back to the tank from which it originated.

The electric pulse was applied once to the individual fish at random position. Grid markers were added to the circumference of the tank (not visible for fish) to enable a quantification of behaviour from video recordings. Prior to exposure, during and afterwards exposure the behaviour was monitored by a video camera and recorded for analysis of behaviour.

Except for the presence of electrodes and the cotton light diffuser on top of the tank to reduce light scattering and hotspots, the conditions were the same as in the holding tanks. When the experiment was finished fish were placed in the tank from which they were caught. This was done to avoid changes in conditions.

A second experiment was carried out on two groups of catsharks on 09/10/2007, a group (9 individuals) to be exposed to an electrical stimulus and a control group (11 individuals). The exposure group and the control group were transferred from two holding tanks to the experimental tank subsequently in which centre a single pair of electrodes was placed. The exposure group was stimulated during 1 s with a reference pulse train of which video footage was recorded. After transferring both groups back to their original tanks their behaviour was observed and recorded at various intervals.

#### Ethical issues

Prior to the start of experiments with live fish a project plan was presented to a governmental ethical committee for approval. As soon as the plan was approved, the study was carried out by scientists and technicians who have a licence for experiments with live fish.

### Results

Measurements on electric fields in a laboratory and *in situ* onboard MFV "Lub Senior" (UK153)

The dimensions and materials of electrodes of pulse simulator were identical to the commercially used system onboard UK153. The highest electrical field strength was proportional to the specific conductivity and was 176 V/m in the centre of the conductors positioned at references 62 mm from the conductors. The ratings varied approx 10% per measurement session under influence of the height of the conductors relative to the bottom surface.

The measurements show that the main parameters of the electrical stimuli produced by all tested systems were electrically equivalent and parameters were reproduced each session in the reported time period.

#### Study into spinal damage in cod (*Gadus morhua* L.)

From the X-ray scans of the total of 25 fish, two fish with a dislocated spinal cord, six with a deformity in the spinal cord attributed to natural causes, and two animals without any dislocation or deformities were selected for X-ray photography. The dislocations could not be observed visually from the outside without using the X-ray technology.

#### Indicators to monitor welfare during husbandry of small spotted catsharks

Prior to the experiment with individual small-spotted catsharks, the animals had been kept for approximately seven months in the marine recirculation aquaculture system at IMARES. A dietary supplement was added to the feed, as recommended by the Harderwijk Marine Mammal Park. We observed that no thyroid problems occurred.

During these seven months the fish were not weighed to analyse growth. We observed that some fish produced eggs. Only during the first weeks after arrival 1 fish out of 23 animals died. The low mortality suggests that the husbandry conditions were satisfactory for this species. A number of behavioural indicators was defined to describe the welfare of the animals under captivity (Table 2).

Table 2: Indicators to monitor welfare during husbandry

Small-spotted catshark	Date and time of
	observation
	(fixed prior or
	after feeding)
Normal behavioural characteristics:	(tick a cross
	below)

Animal with bent spine (head up) on bottom

Swimming with spread fins

Animals in groups on the bottom

Individual sharks have not been lying separately from individuals on the bottom for 3 days or

more: if not, this may suggest problem

Animal approaches offered feed: all of all fish start to swim in response to offered feed

Animal is feeding on the bottom

Breathing in by opening its mouth and breathing out through gills

Frequency of breathing around 60/min

Pale outer appearance (compared to other individuals) may suggest problem

Bright eyes which are not bulging

Abnormal behaviour is absent such as: cork screw-like movements

Burst of swimming movements should be absent.

Fish is not skinny

No bulging out of tissue

No other deviations

Fish approach offered feed rapidly, swimming across the tank

Colour of the skin is steady

Eyes of the shark are not closed

Register % of the animals that swim

Register % of the animals that are resting on the bottom

Experimental design to asses effects of exposure to the electrical field of small-spotted catsharks.

Based on the outcome of the first experiment with the two tagged fish, the following experimental design was drafted for experimentation with the catsharks to assess effects in behaviour of a group of small-spotted catsharks due to exposure to the electric field (Table 3).

According to this design an experiment will start by recording foraging behaviour on video (day 1). On day 5, one day prior to the start of the experiment the baseline behaviour of the fish will be recorded on video. On day 6 the control group will be transferred to the experimental tank. After a video recording period of 2 hrs the animals will be transferred to the holding tank from which they originate. Subsequently, behaviour during feeding will be

recorded 1, 24 and 48 hrs after the last transfer. For the group that will be exposed to the electric field the same procedure of handling and observation should be followed, except for, of course the exposure to the electric field.

In the experiments we monitored whether any deviations from behaviour occurred, i.e. vigorous swimming, foraging behaviour and convulsions.

Table 3: Draft experimental design for behavioural observations of small-spotted catsharks

Small-spot	ted catshark (wild fish allowed to acclimatise to holding system for at least one month)
Day	Activity
1	Foraging of fish in the holding tanks is recorded on video
5	Fish in the holding tanks are recorded on video for 10 min during 2 between 9 and 17 hrs
6	08.00 am: Control group (n $\simeq$ 10) is transferred to experimental tank
	10.00 am: Control group is transferred to tank in which they are kept
	Video registration between 8 and 10 am of fish in experimental tank
	11.00 am: Feed is offered and behaviour is recorded on video
	$10.15$ am: Group (n ${\sim}10$ ) for exposure to electric field is transferred from other holding tank to
	experimental tank
	11.15 am: Exposure to electric field
	12.15 pm: Animals transferred to holding tank in which they are kept
	13.15 pm: Feed is offered and behaviour is recorded on video
7	10.00 am: Control group is fed and behaviour is recorded on video
	11.15 am: Stressed group is fed and behaviour is recorded on video
8	10.00 am: Control group is fed and behaviour is recorded on video
	11.15 am: Stressed group is fed and behaviour is recorded on video
On Day 6,	7 and 8 mortality in both groups is registered
Feed is no	t changed during the trial

#### Assessment of exposure of small-spotted catsharks to the electric field

The fish in the second experiment exposed to the stimulus were dispersed on the bottom of the tank with one individual swimming above the electrodes during exposure. Only this animal showed a response with muscular convulsions. Responses in other fish seem to be absent.

Both the control and the exposed group were fed 1.5 hrs after their transfer to the holding tank from which they came. Deviations in behaviour of both groups could thus be observed. Where normally they were eager to seek the feed, they showed hardly any reaction nor did they take the feed. Observation of feeding behaviour was repeated 3 x 24 hrs later with repeated reluctance to take the feed. The reactions were weak although the exposed group showed a weaker swimming response that the control group. After 48 hrs both groups started eating again, but not vigorously, and with stronger reactions in the control group (Table 4).

Table 4: Overview of reactions of groups of catsharks in experiment 2.

Date	Activity	Exposed group	Control group
09/10/2007	Transfer to experimental tank, one	Little reaction during	Not exposed, but also no
	group exposed to electric field, the	exposure, only by one fish	visible response to
	other not, both groups put back in	above the electrodes. No	offered feed.
	holding tanks, and fed after 1.5 hrs	visible response to offered	
		feed.	
10/10/2007	Feeding after 24 hrs	No increased swimming as	Slightly increased
		response to offered feed,	swimming as response to
		nor taking the feed, no	offered feed, no feeding
		feeding was observed.	was observed.
11/10/2007	Feeding after 24 hrs	Sitting on the bottom while	More active swimming of
		food is offered for about 2	the fish compared to the
		minutes. Only one fish took	exposed group, some
		some food.	feed taken.
12/10/2007	Feeding after 24 hrs	In progress.	In progress.

### Discussion

Measurements on electric fields in a laboratory and in situ onboard MFV "Lub Senior" (UK153)

It should be noted that we succeeded in simulating the *in situ* stimulus measured onboard MFV "Lub Senior" UK153 in our tank experiments using a provisional pulse system and a copied section of the commercially used electrode system all provided by Verburg-Holland Ltd.. The highest electric field rating of 176 V/m measured in the Verburg-Holland reference basin was related to the relatively high specific conductance. The actual electric field strength at sea can be extrapolated from the specific conductance (48.7 mS/cm) measured during the field tests onboard MFV "Lub Senior" UK153, and from the measurements on the same systems in the reference basin.

For an optimal impact of the electric field on flatfish, constant bottom contact of the electrodes is a prerequisite. Various provisions (including a relatively low towing speed) have therefore been made by the manufacturer to achieve this. From an earlier type of electrodes, video recordings exist showing their performance under fishing conditions. Such recordings are not yet available for the electrodes presently in use. Further observations and measurements on this important point are therefore recommended.

#### Study into spinal damage in cod (*Gadus morhua* L.)

In the present experiment only a group under treatment, *i.e.* exposed to the electric field, was studied. To avoid drawing conclusions about the effect of the electrical field that may have been caused by other variables *e.g.* mechanical interaction, a control group is also needed. Catches of cod from a conventional beam trawl gear still

have to be collected and analysed the same way. We observed that 2 out of 25 fish had a dislocated spinal cord. This internal damage could not be observed visually. In addition, in 6 out of 25 fish deformities by natural causes were observed. Due to the rather common occurrence of spinal deformations also by natural causes, it is not clear yet whether possible spinal damages by electric pulses will be lethal to this species. It must be noted also that cod are a fish species with a swim bladder that, caught by any bottom trawl, have little chance of survival due to decompression when heaving from the sea bed. The extent of the electric field outside the pulse trawl is limited. The electric field strength outside the trawl at a distance of more than 20 cm is negligible.

It is foreseen that data collection on board of commercial vessels can be biased, as the crew may have unintentionally selected high quality fish. The handling onboard was not observed. We are aware that handling itself may have been another cause of damage to the fish. Therefore, we would like to extend our trials by exposing live cod in an experimental tank at IMARES to the pulsed electric field and analyse the fish afterwards by X-ray scans.

#### Indicators to monitor welfare during husbandry of small-spotted catsharks

The available indicators appear to be adequate to monitor and control husbandry conditions for this species, as only 1 out of 23 animals died during the past seven months. Moreover, this animal died in the first weeks after arrival of the fish at our institute. We therefore assume that this mortality may be related to the catching process.

Little could be found in the literature on husbandry of small-spotted catsharks. The existing knowledge from the Harderwijk Marine Mammal Park was based on facilities of much larger dimensions, offering more swimming space to the fish. Therefore ample time was allocated to test these guidelines for keeping and treating the sharks in our smaller tanks.

# Experimental design to assess effects of electrical stimuli in behaviour of small-spotted catsharks

The experiment in which we exposed two groups of catsharks to the electric field revealed that handling the fish affects feeding behaviour to such an extent that the effect of the electrical stimulus can not be clearly distinguished. We therefore think of changing the experimental design in such a manner that transferring fish from a holding tank to an experimental tank will be avoided, *e.g.* by placing a pair of dummy electrodes and a pair of pulsed electrodes in two separate tanks, thus treating both groups similarly except for the electrical stimulation.

Another point to discuss is the exposure of the fish in the experimental tank. In further experiments we may determine the threshold distance from the electrode array for a first response. With the measurements on the stimuli in the three systems we made an attempt to ensure that the stimulus applied is a good representation of what can be expected *in situ* at sea. The single pair of electrodes used in the behaviour experiment was triggered with a pulse with shape and basic electrical parameters electrically fully equivalent. The electric field, however, is by nature of this construction not homogeneous. During our experiment the fish were dispersed at different distances and at different orientation to the electrodes, which means that the exposure to the stimulus is not equal in all the individuals. The electrode configuration in the tank left areas where the field strength was practically zero. Fish in these areas were hardly stimulated, which may cast light on the effects of the gear outside the normal working range. It appears that these effects are very low of non-existent.

In reality fish experience differing exposure to the stimulus, as they enter the moving gear and may pass the electrodes quickly when dropping backwards to the codend or be exposed for a longer time when swimming along inside the gear above the electrodes. We are still trying to solve this problem, possibly by quantifying behaviour, and using statistical techniques. Another possibility to consider is to try manipulating the fish in the electric field closer to the electrodes, but this may evoke additional stress reactions.

The basic limitations of a tank experiments are: the stationary condition of the electrical stimulus in relation to the fish, whereas at sea the system is towed with a speed of ~4 kts, the influence of water pressure on the behaviour of the animals, different light and salinity conditions during the experiments and the actual situation at sea. A difficulty is also that repetition of exposure experiments on the same group of fish may introduce behavioural changes e.g. by learning. What we can say is that no fish was affected to such an extent that in the observation period afterwards increased mortality occurred.

# (First) conclusions and recommendations

Measurements on electric fields in a laboratory and *in situ* onboard MFV "Lub Senior" (UK153)

The results of measurements conducted on all available systems showed that the parameters of the electrical stimulus were electrically equivalent under fixed conductivity conditions and were stable during the time period of the measurements.

The final measurements of the commercial system used onboard MFV "Lub Senior" UK153 showed that all other measured systems were electrically equivalent to this result and, as this final measurement also involved the complete electric winch system.

Compared to the electrical stimulus IMARES used in the 1970s and 1980s the electrical stimulus of the Verburg pulse trawl system can be characterized as follows:

- A very low amplitude<sup>1</sup>;
- Slow rising/falling edges of the pulse; due to inductive couplings;
- A 4-5 times higher repetition rate;
- An efficient electrode system with isolated sections;
- A 2.7 times lower input power.

The pulse system is electrically isolated from the main input power circuit. This is an important issue in the overall safety on board of sea-going vessels equipped for electrical fishing.

#### Study into spinal damage in cod (*Gadus morhua* L.)

The analysis of X-ray photographs revealed that 2 out of 25 fish had a dislocated spine. In addition 6 animals out of the group of 25 showed deformations which can be attributed to natural causes. Although the sample size is

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<sup>&</sup>lt;sup>1</sup> Please note that numerical data concerning the exact stimuli are confidential and not given here.

small any effect from the pulse stimulation can not be ruled out, but it still needs to be compared to fish caught with the conventional system. Therefore any definite conclusions can not be drawn at this stage.

In order to establish the relationship between exposure to a pulse electric field and the occurrence of spinal dislocation further research is necessary. It is recommended that tank trials on live fish should be carried out, as experimental conditions can be controlled and, where necessary, fixed. In addition, bias during sampling in a commercial setting can be prevented. Afterwards the fish can be killed by anaesthesia in MS-222 and analysed by X-ray scans.

#### Assessment of effects of electrical stimuli in behaviour of small-spotted catsharks

At this stage no conclusions regarding catsharks can be drawn, but we did not see any mortality 72 hrs after exposure.

We recommend to avoid transferring fish from one tank to another, and ensuring that the exposed group and the control group only differ in treatment by applying the electrical stimulus, *e.g.* by placing a dummy pulse simulator in the control tank. In addition we think of improving our methodology further by monitoring salinity and temperature more frequently, improving contrast of the layer of sand on the bottom of the tank, and improving the light regime. We should also consider effects of multiple exposure to pulse electric fields on the selected fish species, as this is likely to occur in practice when a pulse trawl is used on a commercial scale in practice.

#### General comments

The selection of a limited number of fish species (including benthos) implies that effects of using pulse trawls on other aquatic organisms are not determined. A more integral approach to establish effects of pulse trawls on the marine ecosystem may be warranted, but the questions raised by ICES do not specify exactly which of the ecosystem components need to be addressed, a point that was raised in the discussion in ICES WGFTFB. Additional laboratory trials and field trials may be required for this integral approach.

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# Referees and Authors

Rapport Project Number:	C098/07 4391500101 (DEGREE R%D)	
	en professionally prepared by Wageningen IMARES. The scientific validity of this report has ed and verified by another researcher and evaluated by the Scientific Team at Wageningen	
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