The effects of electric pulse stimulation on cultured adult cod, continued research 2013

D. de Haan, M. Soetaert [1,2] and B. Verschueren [2]

Report number C183/13

1Department of Pathology, Bacteriology and Poultry Diseases, Faculty of Veterinary Medicine, Ghent University,
Salisburylaan 133, B-9820, Gent, Belgium;
2Institute for Agricultural and Fisheries Research (ILVO), Animal Sciences - Fisheries, Ankerstraat 1, 8400, Oostende, Belgium

IMARES Wageningen UR

(IMARES - Institute for Marine Resources & Ecosystem Studies)

Client: Dhr. C. J. M. Verbogt
Ministry of Economic Affairs (EZ)
Prins Clauslaan 8,
2595 AJ Den Haag

This research is performed within "Beleidsondersteunend onderzoek (BO)" of EL&I-programs
BAS-code: BO-12.04-001-025

Publication date: 16-12-2013

IMARES is:

- an independent, objective and authoritative institute that provides knowledge necessary for an integrated sustainable protection, exploitation and spatial use of the sea and coastal zones;
- an institute that provides knowledge necessary for an integrated sustainable protection, exploitation and spatial use of the sea and coastal zones;
- a key, proactive player in national and international marine networks (including ICES and EFARO).

P.O. Box 68	P.O. Box 77	P.O. Box 57	P.O. Box 167
1970 AB IJmuiden	4400 AB Yerseke	1780 AB Den Helder	1790 AD Den Burg Texel
Phone: +31 (0)317 48 09 00	Phone: +31 (0)317 48 09 00	Phone: +31 (0)317 48 09 00	Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 26	Fax: +31 (0)317 48 73 59	Fax: +31 (0)223 63 06 87	Fax: +31 (0)317 48 73 62
E-Mail: imares@wur.nl	E-Mail: imares@wur.nl	E-Mail: imares@wur.nl	E-Mail: imares@wur.nl
www.imares.wur.nl	www.imares.wur.nl	www.imares.wur.nl	www.imares.wur.nl

Contents

Summary

The IMARES study of 2010 on cultured adult and juvenile cod showed that vertebral injuries occurred in 50-70 % of the adult cod at pulse amplitude of 30 to 60 V (de Haan et al., 2011). No injuries were observed in juvenile cod. In a similar ILVO-study on adult cultured cod conducted in June 2013 in Tromsø, Norway no injuries were observed at pulse settings similar to the IMARES application of 2010 and at settings twice the IMARES maximum amplitude rating. To investigate the origin of the different outcome an experiment with the ILVO and IMARES systems was conducted in the period between 9 and 14 October 2013 using the cultured adult cod from the IMR Austevoll hatchery, with which vertebral injuries were observed. Both IMARES and ILVO pulse generators were equivalent to the equipment used in the earlier studies.

In this experiment a total of 83 adult cod were exposed, 53 specimen were exposed at 60 V and 30 specimen twice above the maximum commercially applied amplitude (120 V). In 5 cases vertebral injuries occurred of which 4 at 120 V and a single one at 60 V. When the type of stimulus is taken in account this single injury at 60 V amplitude represents an injury rate of 4.5 % of 22 exposed fish and 13 % at 120 V (4 out 30 specimen). Despite the same electrical parameters were set, none of the 21 fish exposed to the ILVO pulse generator was injured. All injuries occurred using the Delmeco TX68 pulse shape.

The post exposure reaction of the fish exposed at 60 V amplitude seemed less strong than in the IMARES study of 2010. In this study some of the fish accelerated out the holding cage into the main tank, while this did not occur in the present study. A second observation was weaker tail marks indicating vertebral injury, while the fish of 2010 with similar injuries had very strong and large tail marks. The post exposure reaction of the fish exposed at 120 V included electro-narcosis and some became stunned. After dissection parts of the fish were taken to the University of Ghent for detailed analysis on the morphology of the dissected fish.

The present results confirmed the recent ILVO outcome of 2013 and showed that the origin of the conflicting outcome must have been related to differences in morphology of the fish. Vertebral injuries could have been related to deviations in the vertebrae related to unknown changes in the methods of the culture, which could have caused differences in the muscular system, or mineral content of the vertebrae. However the mineral content is expected to be similar for all fish of Austevoll, as all fish used in the experiments between 2008 and 2013 from the Austevoll hatchery had the same exclusive rearing history, in which larvae are fed with zooplankton in the first stages of the culture. The fish used in the Tromsø experiment were reared according the intensive rearing technique, which involves nutritionally enriched rotifers (*Brachionus plicatilis*) and brine shrimps (*Artemia salina*) in the very early stadia of the fish. This seems to exclude a particular rearing method as the minor number of injuries observed in the present study is related to fish reared according the exclusive method.

Seasonal effects in the cultured fish could be excluded as the present study was executed in the same period as in the 2008 study and a month earlier than the study of 2010.

Adult cod can get injured in electrified beam trawls as observed in the landings in the past years and this is a sensitive aspect in the legislation and perception of this new technique. Continued research could focus on sampling the occurrence of vertebral injuries in cod in the commercial landings and of catches of the observed discard programme and on discriminating a specific seasonal relationship and on analysing fish' morphology related to the injury.

1 Introduction

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. Following the ICES Advice on Pulse Trawling on flatfish of 2006 research was carried out by IMARES, the Netherlands on cod (*Gadus morhua* L.) (de Haan et al., 2009a and 2011) and cat sharks (*Scyliorhinus canicula* L.) (de Haan et al. 2009b) and a range of benthic species (ragworm (*Nereis virens* L.), common prawn (*Palaemon serratus* L.), subtruncate surf clam (*Spisula subtruncata* L.), European green crab (*Carcinus maenas* L.), common starfish (*Asterias rubens* L.), and Atlantic razor clam (*Ensis directus* L.) under pulse stimulation of the Verburg-Holland electric pulse system (Van Marlen et al., 2009). These reports were reviewed in 2009 leading to a new ICES advice.

The experiments on cod of 2008 and 2010 were conducted at and in cooperation with scientists of the research station Austevoll of the Institute of Marine Research Bergen, Norway.

The experiments on dogfish and benthic species, executed at the Imares laboratories, showed hardly an effect, the strongest effects were measured in the experiments on cod. The fish exposed with nominal pulse settings in a very short range were injured and suffered haemorrhages close to the vertebral column.

In February 2010 the outcome of the three studies was discussed at the ICES WKPULSE workshop (ICES, 2010) with international experts and the group advocated continued research with the following terms of reference:

a. To review current technical developments on electrical fishing (with main focus on marine systems).
b. To review studies on the relationship of pulse characteristics (power, voltage, pulse shape) and thresholds in effects (mortality, injury, behavioural changes).
c. To improve knowledge about the effects of Electrical Fishing on the marine environment (reduction of bycatch, impact on bottom habitat, impact on marine fauna, energy saving and climate related issues).
d. To evaluate the effect of a wide introduction of electric fishing, with respect to the economic impact, the ecosystem impact, the energy consumption and the population dynamics of selected species.
e. To consider whether limits can be set on these characteristics to avoid unwanted effects (e.g. unwanted and uncontrolled growth on catch efficiency, unwanted ecosystem effects) once such systems are allowed and used at wider scale.

WKPULSE recommended to collect more information on the effect on cod before the pulse trawl can be allowed, and among other items to:

1. Monitor the current (by)catches with the latest version of the system (TX68) and compare these with a conventional beam trawler
2. Sample cod from current (by)catches with the latest version of the system (TX68) and compare the occurrence of spinal damage with those from a conventional beam trawler
3. Investigate the effect on fish not necessarily caught in the trawl after being subjected to the pulse system and determine whether this is a source of unaccounted mortality.

In 2010 a second experiment on cultured cod was conducted at the IMR-Austevoll research station. The main goals for this experiment was to expose juveniles and adult cod in a range of pulse settings to observe a specific pulse parameter related to the vertebral injuries observed in the study of 2008.

The main outcome of this study was that 60 to 70 % of the adult cod (length range 0.34 – 0.56 m) were deadly injured even when the amplitude was reduced with 50 %. When the pulse frequency was increased the number of injuries reduced. At 100 Hz 40 % of the fish were injured and at 180 Hz the injuries reduced to zero.

No injuries were observed in juveniles (length range 0.11 – 0.18 m) even when exposed in very close range of a conductor and highest amplitude settings. The electrode distance applied in this present study was set to 0.325 m, similar to the Delmeco UK153 concept (Table 1) setting and used in the earlier IMARES and ILVO studies.

Table 1 shows the overview of the main pulse settings of the two commercially exploited systems in the Dutch flatfish fisheries and the development since the first laboratory study on adult cod in 2008. The gear of the Delmeco TH10 concerns a 7 m beam length, all others 12 m. The overview shows that since 2008 the electric exposure in terms of pulse amplitude and electrode distance reduced. To be able to relate the outcome all experiments were conducted with the 60 V amplitude and 0.325 m as nominal condition. At present the pulse characteristics of 2010 are still valid.

Table 1: Overview of commercially applied pulse parameters of commercial pulse systems between 2008 and 2010.

Pulse concept	Power Single gear (kW)	Electrode Voltage ($V^{0\ to\ peak}$)	Pulse Freq. (Hz)	Pulse duration (width) (µs)	Electrode		
					Nr	Distance (m)	Conductor (nr) (l x d (mm))
Delmeco UK153 2008	8.5 (0.71)	60	40	220*	32	0.325	6 (180x26)
Delmeco TX68 2010	5.5 (0.46)	50	40	220*	25	0.425	6 (180x26)
Delmeco TH10	2.1 (0.3)	50	40	220*	10	0.425	6 (180x41)
HFK TX36	7 (0.58)	45	45	170/50	28	0.415	2 (125x27) + 10 (125x33)

*The pulse duration refers to a single pulse period. The electric power list between brackets is the conversion to power per meter beam length between brackets

1.1 Field strength references in the laboratory and under full scale practice

In the study of 2010 field strengths applied in the laboratory were also referred to the full-scale commercially applied condition with the pulse gear at the seabed. This condition was measured on board the TH10 (7 m pulse trawl) equipped with a Delmeco pulse system. These references relate the pulse settings in particular the dose in terms of field strength of the experiments in the IMR laboratory to what is used in commercial practice with the pulse gear at the seabed. Under this condition the electric field is less dense and penetrates the stratum, while the electric field in the laboratory is compressed by the plastic tank material. The field strength applied in the laboratory under nominal pulse settings was 54 % above the field strength of the commercial fished condition with the gear at the seabed. Field strength measurements on the HFK pulse concept showed that the values measured during the experiments were similar to those measured with the gear on the seabed for both juvenile and adult cod. These measurements showed that the laboratory experiments of 2010 were conducted in a representative range of field strengths and that injuries observed are likely also to occur in practical fishing conditions.

1.2 ILVO research on the effects to cultured cod

Soetaert et al., 2013 described the history of research on pulse fishing, and made an inventory of existing knowledge and knowledge gaps on the impact of electric fishing on fish species. This inventory was used to define further research topics on a wide range of marine fish and invertebrates. A part of this research addresses the effect of pulse stimuli on cultured adult cod in uniform and non-uniform electric fields using similar pulse characteristics tested by the de Haan et al., 2011. In June 2013 the actual experiment was conducted in Tromsø, Norway with cultured cod from a commercial aquaculture facility (Cod Breeding Centre, Tromsø). The outcome of this experiment showed that not a single fish was injured, not even when pulse amplitudes of 120 V were applied. This outcome contradicted the observations of de Haan et al., 2009 and 2011 where 60-70 % of the exposed adult cod were injured. This raised the question whether the origin of this difference is related to a physical difference in the applied pulse technique or changes in the morphology of the fish, in particular the muscular and vertebrae system.
To investigate the background of this different outcome an experiment was carried out using the cultured adult cod from the Austevoll hatchery with both the IMARES and ILVO-equipment. The experiment was acknowledged by the Norwegian Animal Welfare Authority on 17 September 2013.
As an ICES SGELECTRA meeting was planned between 22 and 25 October 2013 there was an urge to conduct the experiment prior to this meeting to report the results and to discuss further steps.
The IMARES part of the study was commissioned by the Dutch ministry EZ (BO-20-010-043).

2 Materials and Methods

The pulse experiment took place in the period between 9 and 13 October 2013 at the IMR-Austevoll laboratory using the fish tanks of similar dimension used in the Imares studies of 2008 and 2010.
All exposures were conducted with an electrode distance of 0.325 m.

2.1 Imares pulse equipment

The Delmeco pulse simulator equipment consisted of a control unit and power module and was identical to the device used in the Imares experiments of 2008 and 2010 (Figure 1). The pulse stimulus was a simulation of the Delmeco-TX68 pulse characteristics comprising of a bipolar pulse of 220 µs pulse width, with a 12.6 ms bipolar interval and a frequency of 40 Hz. The applied amplitude is the maximum rating of 60 $V^{\text{zero to peak}}$. Given the inductor (Figure 1) connected to the output of the generator the front slope of the pulse raised according a power of E-function (Figure 2 and 4). This inductor was also used in series with the ILVO EPLG 2 generator (Table 2, trial 7, 8 and 9) to simulate the Delmeco pulse shape at amplitude of 120 $V^{zero\ to\ peak}$, which is outside the maximum range of the Delmeco generator.

2.2 ILVO pulse equipment

The ILVO pulse simulator comprised of two different generators marked as EPLG (Electro Pulse Lab Generator) 1 and 2. Both modules produce a type of symmetrical bipolar square wave. The output pulse of the EPLG 1 generator is similar to the Delmeco concept (Figure 3) but not inductively coupled and so the stimulus consists of conventional pulse edges based on a square-wave type of pulse with rise/fall time of 50 µs similar to the experiment in Tromsø. The pulse stimulus of the EPLG 2 generator is a recent adaptation producing steeper edges (10 µs). These short rise/fall times, however, could not be developed under practice with the inductance present in the electrode cabling and instead a rise/fall time of 50 µs was produced (Figure 5). Both generators contain two H-bridge type of electronic switches/pulse units, which are connected in parallel. Each module supports a discharge power of 150 V/140 A at maximum, 42 kW (peak), each generator support a power of 150 V/ 280 A. Shortly before the start of the experiments one of the modules of the EPLG 1 generator appeared faulty, this module was disconnected and the ELPG 1 generator was used with a single module. The pulse output of both generators is not directly produced at the output clamps, but a cycle of 2 s is used to measure the conductance as input to the current protection control. This control feature hampered the direct exposure after a fish was positioned and caused faulty triggers as the fish position was not always stable over such a delay. A second flaw to this facility is that the conductance is apparently measured by an AC signal and was received on the hydrophone system used to detect vertebral injuries. This signal that is audible to the fish it could have alert the fish and caused an unwanted conditioning effect. The fact that the fish did not freely position, but was forced towards the required bottom position in some cases can be regarded as a measure with higher impact on the stress condition of the fish shortly before the exposure.

2.3 Conditions, procedures and measurements

2.3.1 Fish size

All fish were transferred from the pond in the fjord to the holding tank on 9 October 2013 a day before the experiments started. The length of the tested cod ranged between 0.30 and 0.45 m (average 0.39 m) with full body weights in the range of 217 to 1220 grams (average 815 grams). The length weight ratio of the fish tested in this experiment did match the conditions of the fish tested in the experiment of 2010 and in Tromsø 2013 although the present length was slightly smaller (Figure 10).
The fish were tested in groups of 10 to 12 individuals and tagged individually after exposure as an aid to the dissection analysis (Table 2).

2.3.2 Positioning of the fish

The cod was positioned in a cage of plastic meshed material used in the earlier Imares studies and positioning according the methods used in the Imares experiment of 2010 (de Haan et al., 2011).
The positioning was lengthwise as in the study of 2010 (de Haan et al., 2011) with the centre axis of the fish 55 mm aside and 80 mm above the conductor (Figure 6). The head of the fish was positioned opposite of the front part of the conductor. In case the fish did not manoeuvre itself in the required position as indicated above, they were gently forced into this position using positioning aids, consisting of a cage of synthetic mesh netting, and the plate of equivalent synthetic mesh material, as used earlier in the IMARES experiments of 2010. Once the required position was achieved the stimulus was triggered.

2.3.3 Pulse and field strength measurements

The pulse output parameters were measured on a 200 MHz LeCroy WaveSurfer 24XS oscilloscope shortly before or after the exposures. Field strength in the defined ranges from the conductors was measured before and after the exposures. A high voltage differential input probe type ADP 305 (SN5069) was used to measure the pulse amplitude and field strength, and a CWT Rogowski 60B current probe (0.5 mV/A) to measure the electrode current. All voltage measurements in this report are referred to the zero to peak voltage of the positive amplitude of the bipolar pulse. Samples of measurement results were stored as JPEG images on hard disc.

Electric field strength was measured alongside the conductor in steps of 32.5 mm using a probe of fixed spacing of 24.4 mm over the sensing tips. Readings were taken along the 55 mm axis aside of -and parallel to- the conductor, 80 mm above the bottom of the tank, representing the position of the longitudinal body axis of the fish (Figure 6).

The overview of Table 2 of main pulse parameters and the highest field strength reading shows that the presently applied exposures increased a factor 1.5 to 2.5 (120 V amplitude) compared to the values measured in 2010 under similar pulse amplitude conditions. Field strength conditions on trial 3 to 5 were not measured. Conditions of trial 3 are equivalent to trial 1. The conditions of trial 4 and 5 are referred to trial 2.

Table 2 Pulse parameters and field strength against the Delmeco TX68 reference measured in 2010.

Trial (nr)	Pulse concept	Amplitude ($V_{0\ to\ peak}$)	Frequency (Hz)	Pulse duration (µs)	Electrode Current (A)	Field strength (V/m)
2010 Reference	Delmeco-TX68 2010	57	40	220	68	102.73
1	ELPG 1	60	40	225	76	134.84
2	Delmeco-TX68	60	40	220	78	158.20
3	ELPG 2	60	40	200	76	not measured
4	Delmeco-TX68	60	40	220	72	not measured
5	Delmeco “steep”	63	40	220	82	not measured
6	ELPG2 “steep”	120	40	250	130	264.75
7	ELPG 2 + Inductor	120	40	250	150	247.7
8	ELPG 2 + Inductor free	120	40	250	150	247.7
9	ELPG 2 + Inductor	120	40	250	150	247.7

2.4 Conditions during the experiment and observations techniques.

2.4.1 Salinity and temperature

Sea water circulated in the tanks on the IMR facility is pumped from the adjacent fjord at a fixed depth of 55 m. The conductivity and temperature of this water were measured daily and are normally stable and deviate in a limited range. The average values for water temperature and conductivity on this present experiment were respectively 7.2 °C and 34.4 ppt (according a hand-held IMR instrument).

As temperature and salinity of the tank water are important references for electric filed conditions and the IMR measurement equipment incidentally used an IMARES Hydrolab type DS5 CTD-sonde was used to record the salinity and temperature of the tank water more frequent daily base. The overview of Table 3 shows the results of the measurements of the present experiment versus the earlier two studies conducted in similar physical conditions. The salinity condition of the present reported experiment in 2013 based on CTD measurements is slightly higher to the conditions measured in the earlier references, which is also confirmed by the higher discharge current measured between the conductors.

Table 3 Overview of the Austevoll tank water conditions during the experiments of 2008, 2010 and 2013.

Experiment	Temperature (°C)	Salinity (ppt)	Instrument	Electrode current ($A_{0\ to\ peak}$)
2008	8.2	32.95	IMR CTD STD204	68
2010	7.5	34.8	IMR handheld	68
2013	7.7	34.7	IMR-handheld	79.2
	7.39	35.06	Imares Hydrolab DS5	

2.4.2 Video observations

The behaviour of the fish during the exposures was filmed underwater using an IMARES black/white bullet camera (bullet type Sony 1/3 inch 0.05 lux). The video signals were recorded using a Sony GVD-1000 video recorder. The sound in air at the water surface of the tank was recorded on one of the audio tracks to synchronise the sound of vertebral injury to the video footage as evidence of such injury. All recordings were stored on digital mini tape.

2.5 Physical effects of the electric stimulus, post mortem research

After the exposure the fish were transferred to a tank to observe the post exposure behaviour and the occurrence of tail marks indicating vertebral injuries.

At the end of the day the fish was euthanized by using an overdose of tricaine/MS 222. The animals were left in the anaesthetic solution for 10 minutes following stopping of the gill opercular movement. Thereafter, the death was physically confirmed by cutting the gill arches. The fish were dissected to observe haemorrhages on the vertebrae and all dissected fish were photographed. Muscular samples and organs were put in small containers filled with formaldehyde and main parts of the fish (filets and skeleton) kept frozen in the IMR main freezer storage.

The fish was dissected shortly after the experiments to observe vertebral injuries indicated by haemorrhages. Of each fish weight and length were measured and gender identified before dissection started. Each fish was labeled with tags used directly after exposure and photographed to ensure a unique identification in the analysis. The vertebral column of the fish was carefully dissected, visually examined for pathology. Per individual fish, muscular tissue of the anterior part of the tail and tissue of heart, gills, liver, spleen, kidney and mid gut were sampled and stored in formaldehyde for histological post analysis. Main filet parts and skeletons were sorted per individual and kept frozen in the IMR main freezer storage and all taken to the morphology laboratory of the University of Ghent.

3 Results

3.1 Overall

The experiments were executed in the period of 9 to 15 October 2013 and the observations are based on 94 specimen, involving 8 trials with adult cod.
The experiments started with trials with a comparison between the equipment used by ILVO in Tromsø (ELPG-1) and the Delmeco TX68 pulse concept used in 2010. The overview of Table 3 shows the results of the first autopsy per pulse stimulus in order of execution.
Under the maximum commercial pulse amplitude setting a single vertebral injury occurred using the Delmeco-TX68 pulse concept and 4 other cases were found at a pulse setting of the high amplitude condition of 120 $V^{\text{zero to peak}}$.

Table 3 Overview of trials, pulse stimulus and the observed impact of the exposures after dissection

Trial (nr)	Pulse concept	Amplitude ($V_{\text{0 to peak}}$)	Frequency (Hz)	Electrode Current (A)	ID (nr)	Number of fish (nr)	Vertebral Injuries (nr)	Remarks
1	ELPG 1	60	40	76	A1-A11	11	0	
2	Delmeco-TX68	60	40	78	A12-A22	11	0	
3	ELPG-2	60	40	76	A23-A32	10	0	
4	Delmeco-TX68	60	40	72	A33-A44	11	1	
	Control Group	n/a	n/a	n/a	A45-A55	11	0	A50-A55 not dissected
5	Delmeco “rectangular”	63	40	82	A56-A65	10	0	Inductors left out
6	ELPG 2	120	40	130	A66-A75	10	0	
7	ELPG 2 + Inductor	120	40	150	A76-A85	11	2	
8	ELPG 2 + Inductor	120	40	150	A86-A90	5	1	Fish not forced in position
9	ELPG 2 + Inductor	120	40	150	A91-A94	4	1	Fish forced

3.2 Direct responses to the exposures

The fish produced a medium to strong contraction during the exposures at nominal settings, but did not respond with a strong startle response after the pulse distinguished similar to the responses observed in the experiments of 2008 and 2010 (de Haan et al., 2009 and 2011). In the 2010 experiment a number of fish accelerated out of the cage used for positioning the fish regardless of the sustained injury.
At the highest amplitude (120 $V^{\text{zero to peak}}$) the fish became stunned and showed a behaviour indicating epilepsy or reaching the electro-narcosis level. After some minutes the fish recovered from this condition.
As in the studies of 2008 and 2010 vertebral injuries became visible by black circular patterns on the tail (Figure 8), but these marks were not as clear as in the 2008 and 2010 studies. During the trials the sound of vertebral ruptures was heart at the side of the tank and on the loudspeaker connected to the recording instruments.

3.3 Post mortem research

The autopsy analysis started with the observations after dissection of the fish and mainly concerns the occurrence of vertebral injury and the associated haemorrhages. An example is illustrated in Figure 9. Further histological analysis on the preserved samples and X-ray photography will be continued in the laboratories of the University of Ghent.

4 Discussion

Adult cod can get injured in electrified beam trawls as observed in the landings in the past years (van Marlen et al., in press) and this is a sensitive aspect in the legislation and perception of this new technique. Apparently this experiment shows that the impact of pulse exposures in a very neat range of a commercially applied electrode system is not an absolute figure and that the impact of pulse systems measured with cultured adult cod can vary between very strong effects, such as observed in 2010, to marginal effects as found in this present experiment. Seasonal effects can be excluded as the studies in 2008 and 2010 and this present study were done in a similar period of the year. The minor impact we found now can only be related to the morphology of the fish and not to the equipment applied, as we have checked its proper functioning thoroughly. Survival of juveniles that escape through the meshes of the trawl after being exposed to electric stimuli in a healthy condition is an issue of high importance. Cultured fish used in the experiment of 2010 was more sensitive to vertebral ruptures, and the background for this cannot be explained in a particular rearing method. Actually we simply do not know at present what the causes are of these differences, perhaps more trials are needed to reveal this.
In marine fish the effects of electric stimuli are proportional with body length (McK Bary, 1956) and so the injuries will be related to the length of the injured fish. The weight/length ratio of the fish used in all experiments is illustrated in Figure 10 and listed in the overview of length and weight of injured fish in Table 4. However the number of injuries is too low to be conclusive, although the single injury at 60 V (trial 4) was close to the maximum length of that group. Length of other injuries follows the average length of the total of all fish (0.39 m).

Table 4 Length and weight of injured fish

Trial (nr)	Stimulus type	Amplitude ($V^{\text{zero to peak}}$)	Length (m)	Weight (grams)
4	Delmeco TX68	60	0.42 (0.44)	963
7	ELPG 2 +inductor	120	0.37 (0.41)	603
7	ELPG 2 +inductor	120	0.40 (0.41)	821
8	ELPG 2 +inductor	120	0.38 (0.41)	658
8	ELPG 2 +inductor	120	0.40 (0.41)	806

Values in brackets are the maximum length size per group

The fish used in the Tromsø-experiments (Figure 10) with 0.7 to 0.8 m length should have been much more sensitive to injuries than the fish used in the present study, given their greater lengths. The culture history of methods applied in the Austevoll hatchery between 2008 and 2013 was checked, and it appeared all fish had a similar rearing history and were all fed with zooplankton in their very early life stages.

4.1 History of the cultured cod and the sensitivity to vertebral injuries

There are two main rearing techniques distinguished in the culture of cod, an intensive or exclusive rearing method. Intensive rearing implies the use of nutritionally enriched rotifers (*Brachionus plicatilis*) and brine shrimps (*Artemia salina*) in the very early stadia of the fish. The first step to investigate the history of the culture confirmed that the fish used in Austevoll has an exclusively cultured background and was reared with zooplankton in the first life cycles. This is in contrast to the fish used for the ILVO experiment in 2013 from the Cod Breeding Centre hatchery in Tromsø, which were reared in an intensive way.

The difference found in the temperature regime in rearing methods differed in the early stages of the larvae could have caused changes in the mineral status and the mechanical strength of the vertebrae to withstand muscular contractions developed by electrical stimulation. The fish used from the Austevoll 2013 hatchery were raised in closed tanks at 12 °C, while the fish from 2008 & 2010 were raised at a higher sea water temperature of 16-18 °C in a small lagoon (Parisvatnet). That may explain the difference in in L-W relationship (Figure 10) and could be an issue to address in understanding the different injury rate of the Austevoll reared fish used in 2008/2010 and in 2013. The fish from the Cod Breeding Centre in Tromsø in 2013 on the other hand were raised in closed tanks with natural sea water, with temperatures ranging from 5 °C (eggs, early spring) to 12 °C (juveniles, late summer). The juvenile fish tested in 2010 was from the hatch of early 2010, while the adult fish tested in that experiment was probably from the hatch of 2008, so minor changes in the morphology between these groups cannot be excluded. However, the juvenile fish were exposed to very high field strength rates of 200 to 250 V/m with zero observed injury. As the impact of electric fields to fish is mainly related to the body length (McK Bary, 1952) minor changes in the morphology attributing to a lower strength of the vertebrae is thought to have a minor effect to the observed juvenile outcome.
Further research is required to investigate the culture in more detail. This is outside the scope of this short-term experiment.

4.2 Mineral contents of the vertebra

Bone tissue or osseous tissue consists of a composite material incorporating the mineral calcium phosphate, and collagen, an elastic protein which improves fracture resistance. The relationship between the ability of the musculature to contract and the ability of vertebra to withstand muscular contractions will most probably determine how electric stimuli with associated muscular contractions affect the vertebral column (*personal communication* Per Gunnar Fjelldal (IMR) 2013). According to IMR specialists the mineral status of the vertebra will influence their mechanical strength and ability to withstand compression. The development of the vertebral column in cod from the very early stages of cod and its functional anatomy is recently published by Fjelldal et al., 2013. Unpublished data on the mineral content in vertebra of wild and cultured cod showed that the mineral content of the vertebral arches of intensively reared cod is slightly lower than wild cod (Figure 11 (*personal communication* Per Gunnar

Fjelldal (IMR) 2013). This indicates that the vertebra of intensively cultured cod could be less resistant against injuries than wild cold. Although the fish used from the Austevoll hatchery between 2008 and 2013 were all reared according the same methods, minor details could have caused changes in the vertebrae system. On the other hand, one would expect more injuries in the intensive reared cod of the Tromsø hatchery. To clarify this, the mineral contents of the vertebrae of fish of both hatcheries can be analysed in the laboratory of the Ghent University, Belgium.
This part could also involve injured fish from commercial landings. This final part of the analysis will be executed in cooperation with ILVO, IMARES and the Ghent University.

4.3 Effects related to the pulse shape

Statistically it will be hard to relate effects to the pulse shape as the numbers of injured fish are few (5) and other factors such as pulse amplitude (60 V and 120 V) and the positioning of the fish were not constant. Nevertheless all injuries occurred with a pulse shape with an inductive front slope according the Delmeco stimulus. Secondly the number of zero injuries of the total of 163 used with the ILVO equipment over all experiments in 2013 (including the earlier experiment in Tromsø) is much higher that the numbers used in this present experiment (21) with this particular pulse shape.
This all suggests that a rectangular pulse shape could have a lower impact. However the delay in the rise and fall times of the pulse mainly depends on the “natural” inductivity in the pulse discharge circuit and a pure rectangular pulse shape will be hard to accomplish and will vary with design changes of electrodes. This was also expressed in the pulse shape of the EPLG 1 and 2 generator. The EPLG 2 generator supported pulse edges of 10 µs in theory, while in practice both generators produced similar pulse edges of 50 µs (Figure 5). As the generator of the HFK-stimulus does not contain inductive couplings, the pulse shape has the lowest rise/fall times. However, the field strength measurements conducted on the HFK pulse gear on board OD17 in 2010 (de Haan et al., 2011) showed that the inductive shape was strongly related to electrode materials, which contains stainless steel enforcements with less copper connections in newer designs. Electrodes with these enforcements were commercially applied after the laboratory experiments on cod in 2010. The HFK pulse shape applied in the experiment of 2010 contained an inductive type of front edge, while the numbers of injured fish using the HFK-stimulus in the experiment of 2010 were not significantly smaller than the injuries observed using the Delmeco pulse shape. This all suggests that rectangular pulses with steep pulse edges could contribute to reduction of injuries in adult cod.

5 Conclusions and recommendations

- In this present study 2 % of the fish became injured at a pulse amplitude of 60 V and 13 % at an amplitude of 120 V. This experiment showed that the lower impact of pulse stimulation to cultured adult cod is not related to the pulse simulator equipment used, but likely to the morphology of the fish. So far it is not clear which condition is the main driver for the result we found now;
- So far the background of this phenomenon is unknown, dissection analysis is still ongoing and can be executed in cooperation with ILVO, IMARES and the university of Ghent;It is recommended to consider catch comparisons of substantial cod catches from different commercial pulse systems (Delmeco and HFK) and reveal possible morphological similarities in injured cod in relation to pulse characteristics. This could also be implemented in the IMARES discard project. Paired fishing technique enables the possibility to compare the injury rate of “inductive” against a rectangular pulse shape.

6 Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 124296-2012-AQ-NLD-RvA). This certificate is valid until 15 December 2015. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2013 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

7 References

McK. Bary, B., 1956. The Effect of Electric Fields on Marine Fishes. Marine Research, No. 1. Scottish Home Department. Edinburgh, Her Majesty's Stationary Office. Edinburgh, p. 32.

Fjelldal, P. G., Totland, G. K., Hansen, T., Kryvi, H., Wang, X., Søndergaard, J. L. and Grotmol, S. 2013. Regional changes in vertebra morphology during ontogeny reflect the life history of Atlantic cod (Gadus morhua L.), J. Anat. (2013) doi: 10.1111/joa.12049.

Haan, de D., Marlen, van B., Kristiansen, T. S., Fosseidengen, J. E. 2009. The effect of pulse stimulation on biota – Research in relation to ICES advice – Progress report on the effects on cod. Imares Report number C098/08.

Haan, de D., Marlen, van B., Velzenboer, I., Heul, van der J., Vis, van der H. 2009. The effects of pulse stimulation on biota – Research in relation to ICES advice – Effects on dogfish. Imares report number 105/09.

Haan, de D., Fosseidengen, J. E., Fjelldal, P. G. 2011. The effect of electric pulse stimulation to juvenile cod and cod of commercial landing size. IMARES report C141/11.

Marlen, B. van, Grift, R., O. van Keeken, M. S. Ybema, R. van Hal, 2006. Performance of pulse trawling compared to conventional beam trawling. RIVO-report Nr. C014/06.

Marlen, B. van, Vis, H. v.d., Haan, D. de, Burggraaf, D., Heul, J. van der, Terlouw, A., 2007. The effect of pulse stimulation on biota – Research in relation to ICES advice – Progress report with preliminary results. IMARES-report Nr. C098/07, 24 pp.

Marlen, B. van, Haan, de D., Gool, van A., Burggraaf, D., 2009.The effect of pulse stimulation on marine biota – Research in relation to ICES advice – Progress report on the effects on benthic invertebrates. Imares report C103/09.

Marlen, B. van, Wiegerinck, J.A.M., van Os-Koomen, E., van Barneveld, E., (in press). Catch comparison of flatfish pulse trawls and a tickler chain beam trawl. Fisheries Research.

Soetaert, M., Decostere, A., Polet, H., Verschueren, B. and Chiers, K. 2013. Electrotrawling: A promising alternative fishing technique warranting further exploration. Fish and Fisheries, John Wiley & Sons Lmd.

Justification

Rapport C183/16
Project Number: 430860101

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved: B. van Marlen (M.Sc.)
Fishing Gear Technology Scientist

Signature: B. van Marlen

Date: 28/11/2013

Approved: Drs. J. H. M. Schobben
Head Department Vis
i.a.
N. Steins

Signature:

Date: 16/12/2013

Appendix A: Figures and illustrations

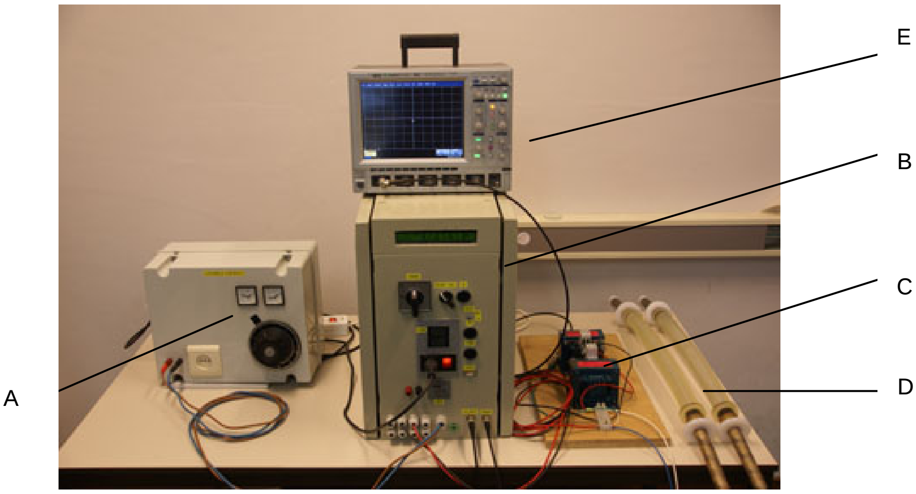

Figure 1: Schematic overview of main components of the simulated Delmeco-TX68 pulse concept (Power module (A), Control unit (B), Inductors (C), Electrodes (D) and (E) the LeCroy oscilloscope.

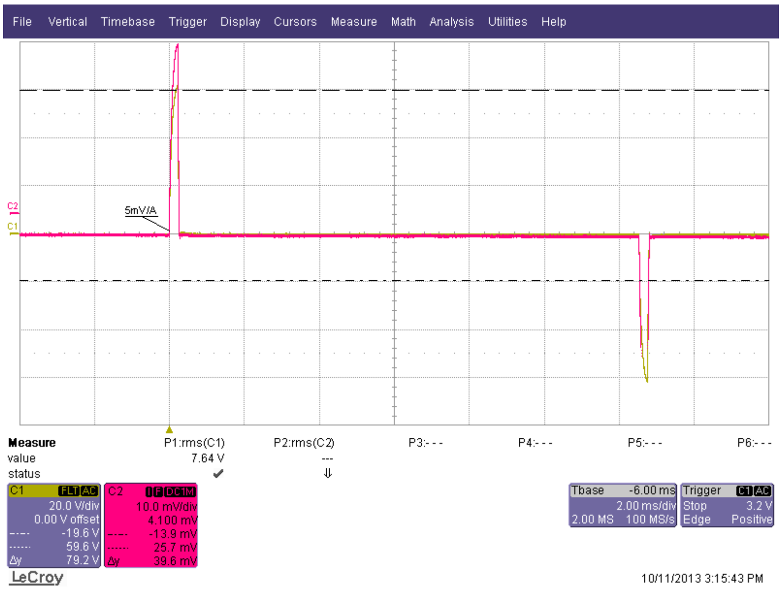

Figure 2: Delmeco-TX68 bipolar pulse concept with inductive shaped pulse edge, 220 µs pulse width, 12.6 ms interval and 40 Hz frequency *(C 1 is the voltage across the conductors (60 V zero to peak), C2 is the conductor current 79.2 A).

Figure 3: ILVO ELPG-2 pulse concept (Table 2, trial 3), marked as "steep" with short rise and fall times (10 µs in laboratory condition).

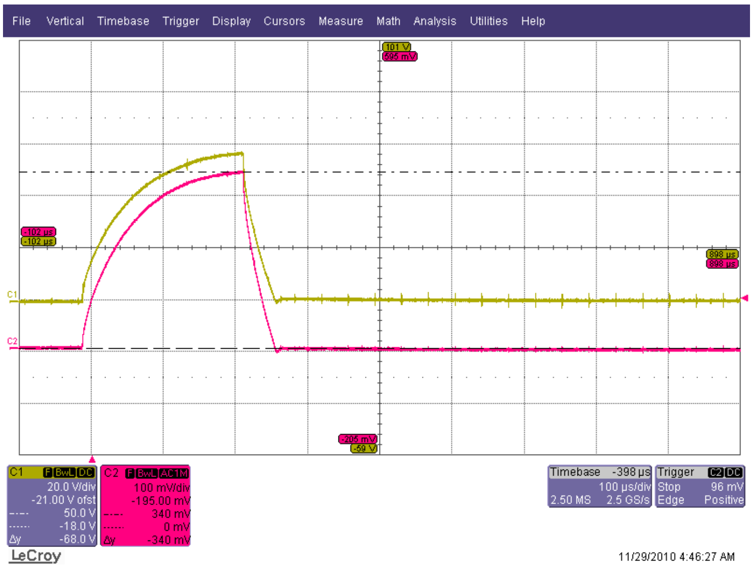

Figure 4: Delmeco TX68 pulse concept measured during the 2010 experiments with 57 V amplitude with the effect of the inductance on the slope of the front edge of the pulse which raises according a power of E function.

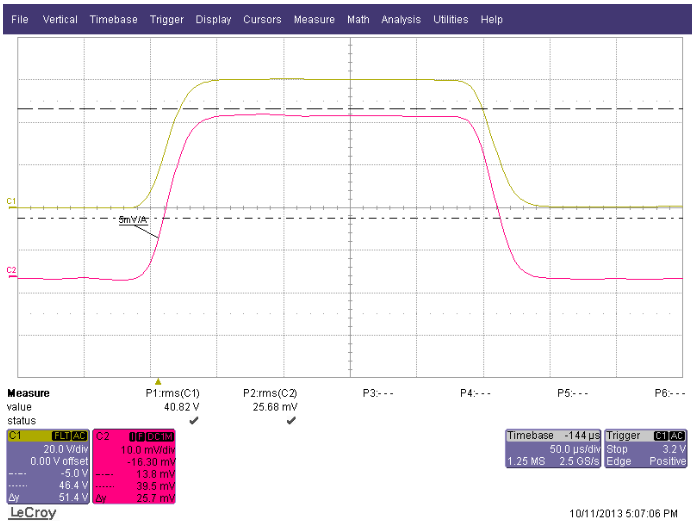

Figure 5: Zoom-in on the ILVO ELPG-2 pulse concept (Table 2, trial 3), marked as "steep" with short rise and fall times. The pulse rise and fall times was 50 µs due to the inductive share of in the discharge part of the electric circuit (cabling) and not 10 µs as specified by the manufacturer.

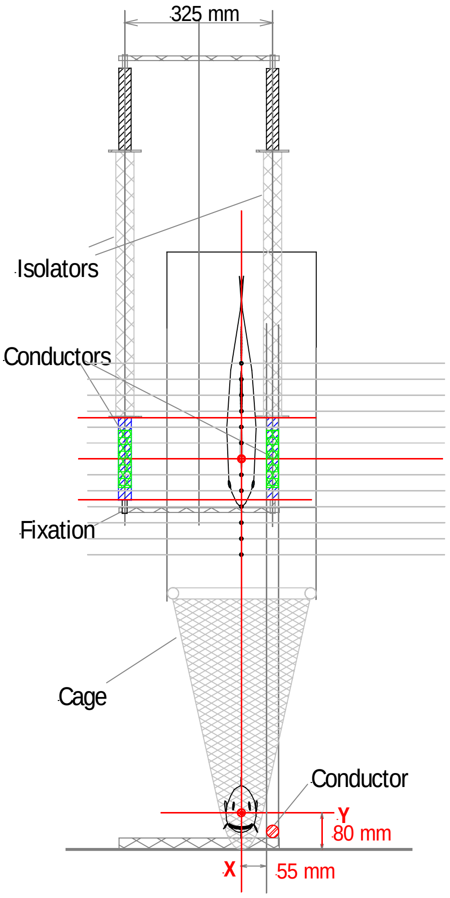

Figure 7: Position of the cod relative to the conductor with the level/distance of the fish body axis as reference for field strength. Values were taken along this axis in steps of 32 mm with the highest value opposite the centre of the conductor (red marked) used as reference for this experiment (Table 3).

Figure 8 Cod exposed in the experiment of 2010 (left) with heavy black tail marks indicating vertebral injury and an example of the present experiment (T35) with similar injury but a less distinctive mark.

Figure 9: Dissected fish including fish T35 (up to the right) with haemorrhage indicting vertebral injury.

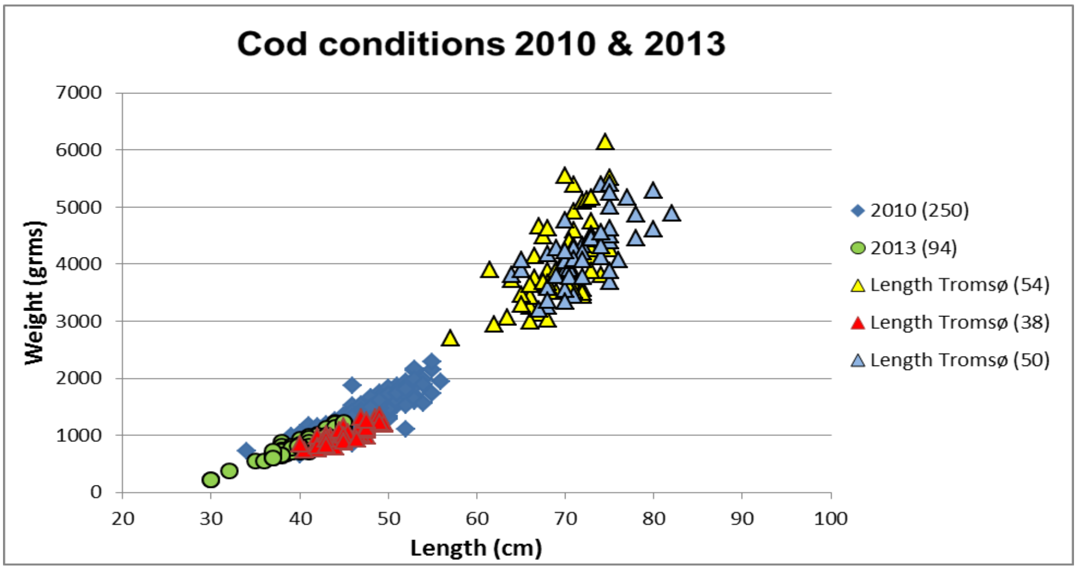

Figure 10: Overview of length/weight ratio of fish tested in Austevoll between 2010 and 2013 and in Tromsø 2013. Values between brackets are the total numbers of exposed fish.

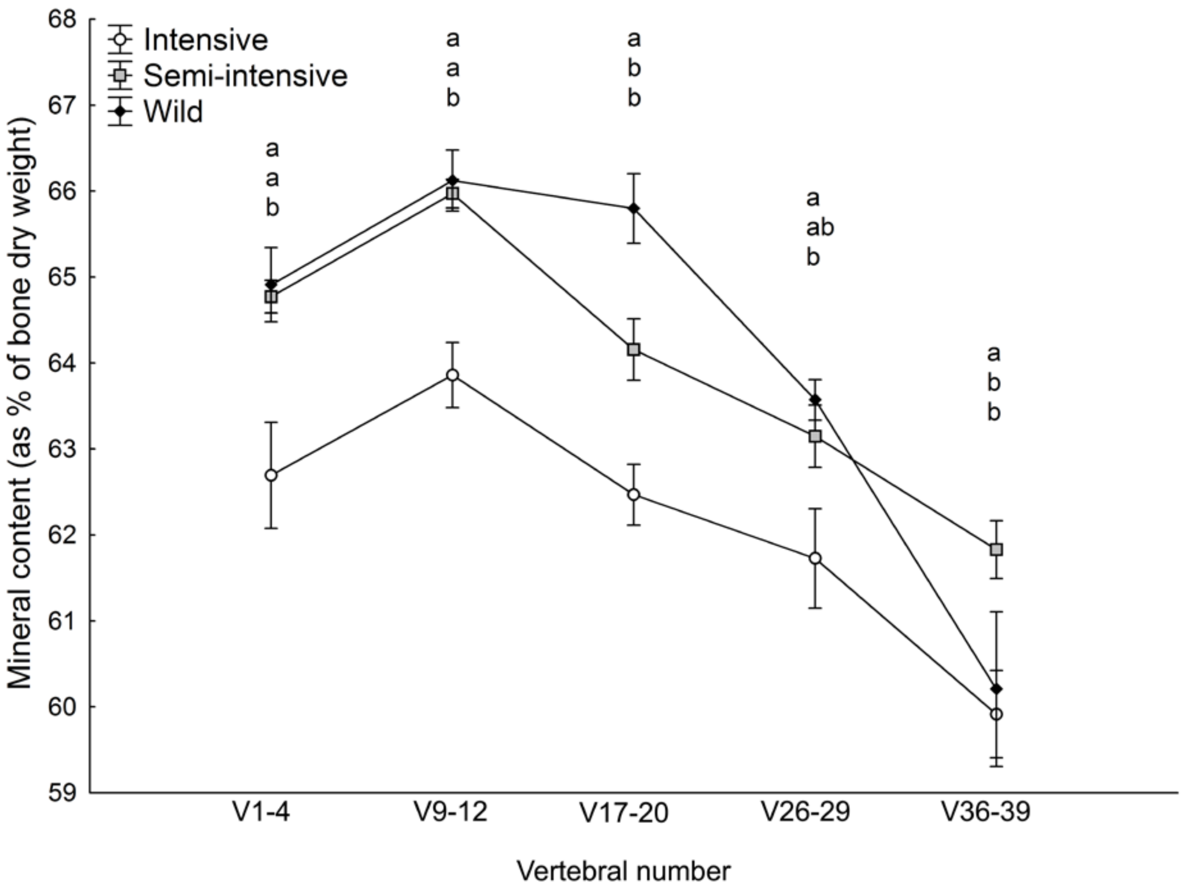

Figure 11: Mineral content of vertebral arches of cultured and wild cod (unpublished data Fjelddal, 2013)