Atlantic Cod Show a Highly Variable Sensitivity to Electric-Induced Spinal Injuries

Author: Soetaert, M.

ARTICLE

Atlantic Cod Show a Highly Variable Sensitivity to Electric-Induced Spinal Injuries

M. Soetaert*

Department of Pathology, Bacteriology, and Poultry Diseases,
Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke,
Belgium and Institute for Agricultural and Fisheries Research, Animal Sciences–Fisheries, Ankerstraat 1,
B-8400 Oostende, Belgium

D. De Haan

Institute for Marine Resources and Ecosystem Studies, Fishery Part of Wageningen UR,
Post Office Box 68, 1970 AB IJmuiden, The Netherlands

B. Verschueren

Institute for Agricultural and Fisheries Research, Animal Sciences–Fisheries, Ankerstraat 1,
B-8400 Oostende, Belgium

A. Decostere

Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133,
B-9820 Merelbeke, Belgium

V. Puvanendran

Norwegian Institute of Food Fisheries and Aquaculture Research AS, Muninbakken 9-13,
9291 Tromsø, Norway

J. Saunders

Department of Veterinary Medical Imaging and Small Animal Orthopaedics,
Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke,
Belgium

H. Polet

Institute for Agricultural and Fisheries Research, Animal Sciences–Fisheries, Ankerstraat 1,
B-8400 Oostende, Belgium

K. Chiers

Department of Pathology, Bacteriology, and Poultry Diseases,
Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke,
Belgium

*Corresponding author: maarten.soetaert@ilvo.vlaanderen.be

Abstract

Pulse trawling is the most promising alternative to conventional beam trawls targeting Sole *Solea solea* (also known as *Solea vulgaris*), but due to the electric fields created by electrotrawls spinal injuries are reported in gadoid round fishes such as Atlantic Cod *Gadus morhua*. This study aimed to investigate the variability in the occurrence of electric-induced spinal injuries in cod. Four groups of cod, each originating from a different wild or farmed stock, were exposed to the pulses used by electrotrawls targeting Sole. Effects were analyzed based on behavior, mortality, and lesions up to 14 d after exposure, and morphological characteristics such as size, somatic weight, muscularity, the number of vertebral bodies, and the vertebral mineral contents of animals were compared among different cohorts. Second, the influence of factors such as water temperature, electrode diameter, and pulse type and amplitude were tested. Electrode diameter and pulse amplitude showed a positive correlation with the intensity of the fish's reaction. However, the present experiments confirmed that cod also show variable vulnerability, with injury rates ranging from 0% to 70% after (almost) identical exposures near the electrode. This indicates that these injuries are not only determined by the pulse parameter settings but also by subtle, fish-specific factors. Although the absence of a sensitive group of cod did not enable the elucidation of the conclusive factor, the effect of physiological and morphological factors such as intervertebral ligaments and rearing conditions during early life merit further attention in future research.

In beam trawl fisheries, tickler chains, chain matrices, and bobbin ropes are used to mechanically stimulate and catch flatfish or shrimp. However, these gears have well-known disadvantages, such as high fuel consumption and seabed disturbance, resulting from their intense bottom contact (Depestele et al. 2016). In addition, beam trawling for Sole *Solea solea* (also known as *Solea vulgaris*) is characterized by poor selectivity because it is a typical mixed fishery with high discard rates. The most promising alternative is pulse fishing, in which the mechanical arousal by tickler chains or bobbins is replaced by electric stimulation with electrodes, inducing electric pulses. In the majority of vessels in the North Sea targeting Sole, a bipolar cramp pulse of 40 to 80 Hz is used to increase the catch efficiency (Soetaert et al. 2015). In these gears, the removal of the tickler chains reduces the drag drastically, which results in fuel savings of up to 50% and reduced bycatch (van Marlen et al. 2014) as well as decreased seabed impact (Depestele et al. 2016).

Studies investigating the side effects of these electric pulses have revealed varying results for different marine species. Exposure of invertebrates (Soetaert et al. 2014), Sole (Soetaert et al. 2016), and European Seabass *Dicentrarchus labrax* (also known as European Bass *Morone labrax*; Soetaert 2015) to the electric pulses used in the field did not elicit mortality or lesions. Gadoid roundfish such as Whiting (also known as European Whiting) *Merlangius merlangus* and Atlantic Cod *Gadus morhua*, on the other hand, displayed spinal injuries (De Haan et al. 2011; van Marlen et al. 2014; Soetaert et al. 2016), albeit with different levels of severity in terms of the number of animals affected. These spinal injuries resulted from powerful convulsions of the body musculature when fish were exposed to electric pulses (Snyder 2003). Catch comparisons in the field demonstrated spinal injuries in 4 of the 45 cod caught by pulse trawlers (van Marlen et al. 2014), while 70% of the cod (>30 cm) experimentally exposed near the electrodes' conductors to field strengths ≥37 V/m were impacted (De Haan et al. 2011). In the same study, juvenile cod (12–16 cm) exposed to high field strengths of 250–300 V/m did not exhibit vertebral injuries (De Haan et al. 2011). In a recent laboratory study, no spinal injuries were observed when cultured and wild-caught cod were exposed to similar pulses in a homogenous electric field (Soetaert et al. 2016). These results suggest that the extent to which electric pulses exert a negative impact does not depend solely on the electric pulse parameters but may also be affected by fish-specific morphological differences or the experimental conditions (conductivity, electrode configuration, and so forth). Variable rates of electric-induced injuries are also reported in freshwater electrofishing, particularly with Salmoninae (Snyder 2003). Since gadoid roundfish have similarly high numbers of vertebrae (±52), it can be hypothesized that such variability also occurs in cod. Although electrotrawl-induced spinal injuries are a major concern, no research has been done to compare the sensitivity of different stocks of marine species. Moreover, there are no studies correlating morphological and physiological factors with injuries in marine fish species.

The goal of the present study was to assess the extent of variability in electric-induced spinal injuries in Atlantic Cod stemming from the cramp pulse used in commercial electrotrawls. Wild and farmed cod of different stocks with different sizes, morphology, and conditions were exposed to the same electric pulses and electrode setup. Subsequently, behavior, mortality, and lesions were recorded for up to 14 d after exposure. The somatic weight, muscularity, number of vertebral bodies, and vertebral mineral contents of animals from these groups were examined by autopsy to reveal possible correlations with the observed reactions. Additionally, the effects of water temperature, pulse type, pulse amplitude, the fish's body orientation, the presence of an inductor, and the electrode diameter were tested. These environmental factors are also variable in electrotrawls targeting Sole and may therefore provide better insight into how they may result in different injury rates in cod caught by commercial electrotrawls.

Methods

Animals and housing facilities.—To examine the variability in the occurrence of electric-induced spinal injuries among different stocks, wild and farmed Atlantic Cod of different origins were compared. The first group (W) consisted of wild cod (39 ± 5 cm [mean ± SD]) caught with hook and line in the North Sea at the Blight Bank Wind Farm 40.2 km off the Belgian coast. They were housed as described previously (Soetaert et al. 2016) and acclimated for 3 months. A second group (F1) comprised 2.5-year-old farmed cod (40 ± 2 cm) obtained from the Institute of Marine Research, Austevoll, Norway. This group was included as a reference group for the experiments done by De Haan et al. (2011), who performed their experiments at this research station using cod of the same stock. The cod were transferred 2–3 d prior to treatment from sea cages to an open-air circular tank of 6 m³ that was continuously supplied with fresh seawater pumped from the adjacent fjord. This tank was covered with a plastic canvas tipi to reduce the light intensity and minimize external perturbation. No food was provided during the days prior to exposure. A third group (F2) of cod (71 ± 4 cm) was taken from the 4-year-old broodstock of the Norwegian Cod Breeding Centre, Norwegian Institute of Food Fisheries and Aquaculture Research (NOFIMA), Tromsø. This broodstock has been intensively selected during the past decade for commercial farming and may therefore show significant morphological differences from the F1 fish. Moreover, they were much larger than the previous two groups. These animals were kept in sea cages from July through January and then moved to large circular tanks of 23 m³ supplied with fresh seawater during the spawning season from February through June. This group was divided in two subgroups (F2a, F2b). The F2a group consisted of randomly chosen gravid cod of both sexes. Animals of the F2b group were first selected based on tail muscularity, with the tail circumference right before the caudal fin measuring at least 20 cm. All F2 animals were fed daily with dry pellets (Vitalis Cal, Skretting, Spain). The fourth group (F3) of cod (45 ± 3 cm) consisted of 2-year-old offspring of F2 fish and were similar in size to the F1 fish. They were housed at the Aquaculture Research Station (Tromsø, Norway) in circular tanks of 2 m³ continuously supplied with fresh seawater from the adjacent fjord. These fish were fed daily (Amber Neptune 300, Skretting, Averoy). All these farmed animals were intensively reared in tanks during the first 10 months of their life before they were transferred to sea cages. These experiments were approved by the Belgian (ID 2011/170) and Norwegian Animal Welfare and Ethical Committees (ID 5183).

Experimental setup and variables.—Fish from the W, F1, and F3 groups were tagged with individually coded Floy tags at the base of the first dorsal fin. Fish from the F2 group were individually identified for the breeding program by means of PIT tags. In all experiments, animals were taken from a pre-exposure housing tank and exposed individually to an electric field in an identical tank. Thereafter, they were moved to an identical postexposure housing tank and housed together and monitored for 2 weeks, except for the F1 animals, which were euthanized 1–24 h postexposure. During exposure to the electric pulses, the reactions of the animals were recorded with a digital video camera. A total of 25 wild, 80 F1, 27 F2a, 35 F2b, and 38 F3 cod were used, of which 5, 10, 5, 5, and 10 animals were used as controls, respectively (Table 1). The control animals were included to compare possible mortality and histological abnormalities between exposed and nonexposed animals. They were chosen randomly and handled similarly but not exposed to electric pulses.

The experiments were set up similarly for all animals (Table 1) but performed at different times and water temperatures in the same year (2013): group W: December (15.0°C), F1: October (7.5°C), F2a: April (3.5–4.0°C), F2b: June (4.5–4.9°C), and F3: May (4.4–5.5°C) (Table 1). The salinity remained constant at 34‰. All experiments were performed with wire-shaped electrodes resulting in a heterogeneous electrical field, but two different electrode configurations were used. First, the same wire-shaped electrodes of a Delmeco pulse trawl system for flatfish were used as described by De Haan et al. (2011). These consisted of two copper conductors (0.18 m; diameter, 26 mm) lifted from the bottom by two polyvinyl chloride (PVC) discs (diameter = 70 mm, 10 mm width) at both ends and separated by an insulator of 0.57 m, as illustrated in Figure 1. However, the discs were removed during all experiments with F2 and F3 fish (Figure 1). The distance between the electrode cores was 0.325 m. Second, electrodes of the Marelec pulse trawl system for shrimp were used in two experiments with F3 fish (pulse IDs IX and X; Table 1). These consisted of stainless steel with a copper core without any insulation (length = 1 m, diameter = 12 mm). The electrodes were placed at the same distance of 0.325 m, in contrast to the 0.7-m distance used on commercial electrotrawls targeting brown shrimp *Crangon crangon*. To compare the electric fields generated by the two electrode configurations, an approximating simulation of the electric field strengths around and in the cod (assuming a two-dimensional field and constant electric resistance for the fish) was made with Finite Element Method Magnetics (FEMM) (Meeker 2006), a free software package that solves two-dimensional planar and axisymmetric problems in low-frequency magnetics and electrostatics. The electrodes' potential difference was 60 V, while the conductivity of the copper electrodes, the seawater, and the sand was set at 58×10^6, 4.2, and 1 S/m, respectively. A value of 0.0115 S/m was adopted for fish conductivity, as recommended by Miranda and Dolan (2003).

During the reference exposures of all groups (pulse IDs I and II), Atlantic Cod were exposed in the near field configuration as described by De Haan et al. (2011). In this exposure, the cod were placed with their longitudinal body axes as close to the conductor as possible while the tips of their snouts were located at the front of the first conductor by placing the animals in a triangular V-shaped cage made of PVC netting following De Haan et al. (2011) (Figure 1). The bottom of the V-shaped cage was attached near

TABLE 1. Overview of experimental design, pulse settings, and results. All animals were held close to and parallel to the conductors during exposure. Electric stimuli were produced by either the Delmeco generator used on commercial electrotrawls targeting Sole or by the Labo Pulse Generator (LPG). Salinity was 34‰ at all times, while the water temperature (Tw) at the moment of exposure was as recorded. The pulse parameters given are as follows: exposure time (t), frequency (F), peak amplitude (U), peak current (I), pulse type (T), pulse duration (D), rise time (R), duty cycle (dc), and peak power (P). The pulse type was pulsed bipolar current (PBC) or pulsed alternating current (PAC). The percentage of animals that demonstrated epileptiform seizures (epi), paravertebral hemorrhages (hem), and spinal injuries (inj) are listed.

		Setup				Pulse settings										Effect (%)		
Group	Pulse ID	Generator	Inductor	Electrode	Tw (°C)	t (s)	F (Hz)	U (Vp)	I (Ap)	T	D (µs)	R (µs)	dc (%)	P (kWp)	No. fish	Epi	Hem	Inj
W	I	LPG	No	Delmeco	15.0	2	80	60	72	PBC	310	40	2.48	4.32	20	0	5	5
	CTR														5	0	0	0
F1	I	LPG	No	Delmeco	7.5	1	80	60	76	PBC	310	40	2.48	4.58	20	0	0	0
	II	Delmeco	Yes	Delmeco	7.5	1	80	60	78	PBC	270	220	2.16	4.68	23	0	4	0
	III	Delmeco	No	Delmeco	7.5	1	80	60	82	PBC	270	60	2.16	4.92	7	0	0	0
	IV	LPG	No	Delmeco	7.5	1	80	120	150	PBC	310	60	2.48	18.00	10	80	0	0
	V	LPG	Yes	Delmeco	7.5	1	80	120	130	PBC	350	250	2.80	15.60	10	30	30	20
	CTR				7.5										10	0	0	0
F2a	I	LPG	No	Delmeco	3.5	2	80	58	80	PBC	310	60	2.48	4.64	16	0	0	0
	VI	LPG	No	Delmeco	3.5	2	40	58	80	PAC	550	80	2.20	4.64	6	0	0	0
	CTR				3.5										5	0	0	0
F2b	I	LPG	No	Delmeco	4.5	2	80	60	80	PBC	310	60	2.48	4.80	10	0	0	0
	VII[a]	LPG	No	Delmeco	4.5	2	80	60	80	PBC	310	60	2.48	4.80	10	0	0	0
	VIII[b]	LPG	No	Delmeco	10	2	80	60	80	PBC	310	60	2.48	4.80	10	0	0	0
	CTR				4.5										5	0	0	0
F3	I	LPG	No	Delmeco	4.4	2	80	60	80	PBC	310	60	2.48	4.80	14	0	0	0
	IX	LPG	No	Marelec	4.4	2	80	61	165	PBC	260	120	2.08	10.07	7	0	0	0
	X	LPG	No	Marelec	4.4	2	40	60	165	PAC	520	120	2.08	9.90	7	0	0	0
	CTR				4.4										10	0	0	0

[a] Fish exposed perpendicularly with its head above the first conductor and its tail above the other conductor.

[b] Fish acclimated for 14 d at 10°C and exposed at 10°C under pulse ID I settings.
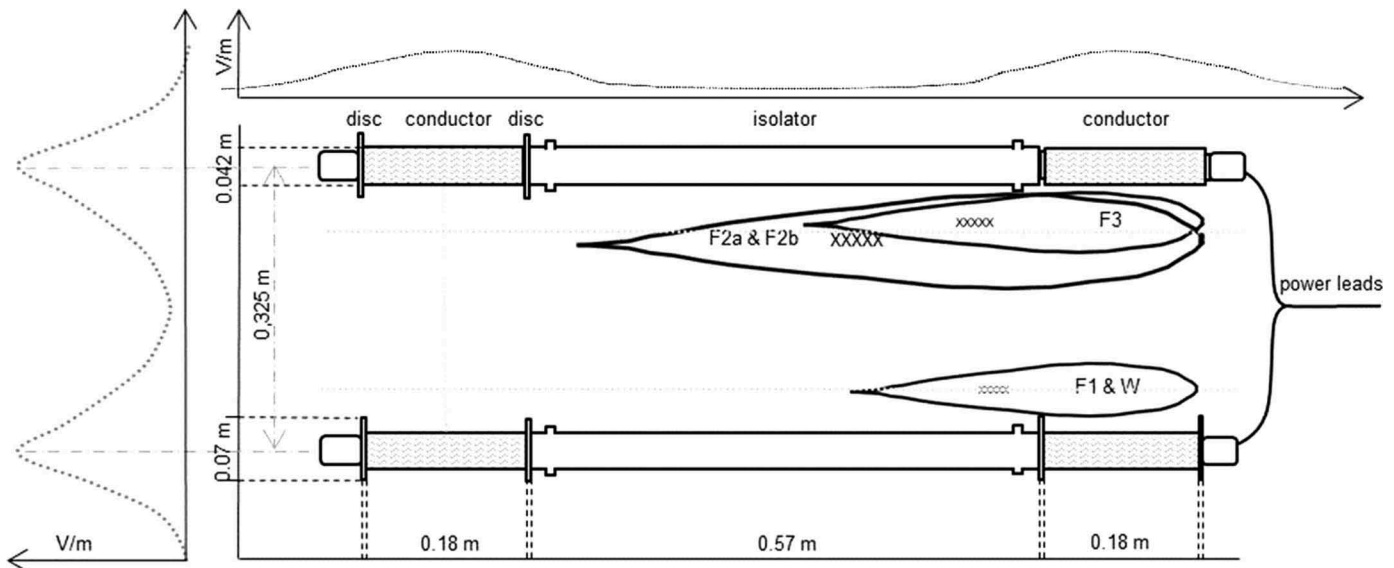

FIGURE 1. Schematic top view of the principal setup with Delmeco electrodes with (lower electrode) and without discs (upper electrode) at both ends of the conductor. The dashed lines indicate the size of each part. The horizontal dotted black graph shows the course of the field strength (V/m) measured at the dotted gray line 55 mm from the electrodes. The vertical dotted black graph shows the approximate exponential course of the field strength measured perpendicular to the center of the conductor. Localization of spinal injuries is indicated with Xs.

the electrode. The fish could swim freely but were forced down-ward using a plate of equivalent mesh material on a stick. Immediately afterwards, the pulse was triggered. As soon as a response by the fish was felt, the fish was no longer forced down-ward to minimize the influence on the reaction during and after exposure (De Haan et al. 2011). In one trial with F2b fish (VII), an exception was made and the animals were oriented perpendicularly with their snouts above one conductor and their tails above the other. In part of the experiments with F1 fish (II, V), an inductor was used in series with the leads to the electrodes to simulate the effect of inductive elements on the discharge circuit (switching transformers, cabling). This inductor prolonged the rise time of the pulse (R [μs], defined as the interval between the onset of the pulse and attainment of its peak amplitude) from 50 to 250 μs.

Pulse parameters.—The amplitude (A [V]) of the pulses was modulated by the pulse generators based on the potential difference over the electrodes. Other pulse parameters were the frequency (F [Hz]), the number of pulses per second; electric current (I [A]), pulse duration (D [μs]), the duration of a single pulse, and the pulse type (T). Both of the pulse types employed in electrotrawls targeting Sole were used: a pulsed alternating current (PAC) with a positive and negative part in each pulse and a pulsed bipolar current (PBC) with alternating positive and negative pulses. The nominal pulse had a square shape and a pulse duration of 250 μs, although this was strongly affected by the impedance of the inductor, electrodes, and power leads. The exposure time was 1 s in the experiments with animals of the F1 group and 2 s in all others, which is approximately twice as long as in the field situation. The duty cycle (dc [%]), i.e., the ratio of the active pulse duration (D [s]) and the period of a pulse cycle (1/F [s]) is given as a measure of the time during which electric current was running. Finally, the maximal electric power (P [W]) released by the electrodes in the water was calculated by multiplying the peak amplitude in volts by the peak current in amperes. An oscilloscope (Tektronix TDS 1001B) was used to measure the effective potential difference, pulse shape, and rise time on the conductors. To determine the electric current, an additional Rogowski shunt was employed. The measured values are listed in Table 1.

Postexposure examination.—After exposure, external lesions, aberrant behavior, and mortality were recorded 1, 2, and 24 h postexposure (group F1) and 1 and 2 h and each 24 h for 14 d (all other groups) by visual inspection of the fish, preferably during feeding. If an injury was suspected, the fish was caught with a dip net and examined more closely. Animals from group F1 were euthanized 2–24 h postexposure, while those from other groups were sacrificed 2 weeks after exposure using an overdose of tricaine methanesulfonate (MS-222). Ten minutes following the cessation of opercular movement, death was physically confirmed by cutting the gill arches and bleeding the animals. At necropsy, the total lengths (from the tip of the snout to the end of the tail; L) and

the whole wet weights (kg) of the Atlantic Cod were measured. All fish were examined for external abnormalities with a special focus on darkening or blackening of the tail region, external bleeding, and wounds. The weights of the gonads, intestinal tract, and left epaxial muscle (W_m) as well as the somatic weight (eviscerated fish; W_s) were recorded. The weight of the gonads was used to calculate the gonadosomatic index (GSI) by dividing the gonad weight by W_s (Lambert and Dutil 1997b). The intestine somatic index (ISI) was determined by dividing the weight of the intestinal tract by W_s. Somatic weight was also used to calculate Fulton's condition factor for the animals, expressed as $K = 100 \cdot W_s/L^3$ (Bagenal 1978). In addition, the muscle factor was calculated as $M = 100 \cdot W_m/L^3$.

Internal organs were also examined for lesions, and samples of the gills, dorsal muscle (base of third dorsal fin), heart, liver, spleen, gut, and kidney were collected and processed for histological examination and compared with the control animals as described by Soetaert et al. (2016). After the autopsy, fish carcasses were labelled and frozen, and lateral and dorsoventral radiographs with X-rays (60 kV, 12.5 mAs) were taken. For the F2 and F3 animals, radiographs were taken at the NOFIMA facility in Tromsø (Siemens Nanodor 2 X-ray machine), while all other radiographs were taken at Ghent University (EDR6 Canon, type CXDI-50G flat panel detector, scintillator, and amorphous silicon Sensor LANMIT 4; Santa Clara, California). All photographs were exam-ined to detect possible malformations, fractures, or luxations. Additionally, the number of vertebrae was determined for a mini-mum of 10 individuals per group.

The 22nd–25th vertebral bodies (the most common region for spinal injuries) of 15, 10, 6, 6, and 6 fish of groups W, F1, F2a, F2b, and F3, respectively, were collected. These verteb-rae were boiled, brushed in running tap water, immersed for 4 d in 100% acetone for dehydration and removal of fat, and dried at room temperature. Thereafter, the 23rd vertebra was detached from the others, dried at 65°C to a constant dry weight, and weighed with a precision of 10 μg (Mettler-Toledo, XP 205). The dried 23rd vertebrae were then inciner-ated for 7 h at 850°C and the ashes weighed (= mineral weight; Sbaihi et al. 2007). The mineral ratio was then calcu-lated as MR (%) = 100 · mineral weight/dry weight.

Statistical analysis.—To accommodate the small sample sizes and high number of zeros (no effect) in the data set, Fisher's exact tests were performed if at least one epileptic seizure, spinal injury, or instance of paravertebral bleeding was encountered. The data were pooled to analyze the effect of four variables: (1) the difference in response after identical exposure (pulse ID I) between wild and farmed fish, (2) the difference in response after identical exposure (pulse ID I) between farmed F1 fish and farmed F2 and F3 fish, (3) the difference in response between F1 fish exposed to 60 V and those exposed to 120 V, and (4) the difference in response between F1 fish exposed to 120 V with and without an inductor.

RESULTS

Behavioral Reactions

Nonexposed control animals showed no or weak escape behavior during and after placement near the electrode. Irrespective of the exposure, all exposed Atlantic Cod exhibited a cramp reaction until the end of the electrical stimulus. During the cramp reaction, the fish's body remained straight and no horizontal or vertical bending was observed, whereas the head was pushed backwards and the opercula were distended. According to the fish handler, animals exposed to the Delmeco electrode exhibited stronger contractions than those exposed to the thinner Marelec electrodes (pulse IDs IX and X), resulting in a faster and more powerful displacement of the stick that was used to position the fish during exposure. To find an explanation for this, we simulated the electric field strength around and in the cod (Figure 2). In the immediate vicinity of the thin Marelec electrodes the field strength was higher, but it also showed faster exponential decay. Consequently, the field strengths inside the fish's body were smaller.

The behavioral reaction immediately after exposure at 60 V was variable. No reaction was observed in 10–20% of the Atlantic Cod, 60–70% showed weak escape behavior and swam away, and 10–30% showed very agitated swimming and occasionally jumped out of the open triangular cage. In general, fish that showed more agitated behavior prior to exposure (e.g., wild cod) exhibited a stronger flight reaction. When the potential difference on the Delmeco electrodes was doubled to 120 V (pulse IDs IV and V), 11 of the 20 animals exhibited epileptiform seizures, which was a significant increase compared with the 0 (of 50) fish exposed to 60 V ($P < 0.001$; Table 2). The highest number of epileptiform seizures was observed in cod exposed to the highest power (pulse ID IV [18 kWp]; Table 1) without an inductor. The number of epileptiform seizures was much lower when an inductor was used and the power dropped to 15.6 kWp (pulse ID V; Table 1), although this difference was not significant. The seizures consisted of myoclonic jerks resulting in a lack of responsiveness during which the fish were lying on their sides and not showing opercular movement. The latter slowly returned after 30–90 s, with these fish showing uncoordinated behavior up to 30 min following exposure and dazed swimming for the first few hours postexposure. This was previously reported during homogeneous exposures between plate electrodes (Soetaert et al. 2016) and when juvenile cod were exposed in a nearly heterogeneous field setup (De Haan et al. 2011).

Physical Injuries

During autopsy, paravertebral hemorrhages were observed in five Atlantic Cod. In four fish (W, pulse ID I; F1, pulse ID II; F1, 2× pulse ID V), a dark discoloration of the skin located ventral to the second dorsal fin was observed immediately following exposure and in one animal (F1, pulse ID V) blood originating from the anal opening was noted (Table 1). Immediately after release into the housing tanks, these animals were able to swim upright but showed less active behavior. When a dip net was placed in the tank, they joined the other fish. The W cod displaying the darker skin coloration swam nearer the surface than the other cod during the follow-up period, but they showed normal escape behavior when a dip net appeared. No abnormal opercular movement or loss of equilibrium was observed.

X-ray analysis disclosed acute spinal injuries in 3 of the 5 fish with paravertebral hemorrhages, located between the 20th and 25th vertebrae. This region was the same for all of the injured Atlantic Cod encountered. The affected W cod showed a complete luxation with associated bone fractures (Figure 3, panel 1C), whereas the two F1 fish had a slight subluxation (Figure 3, panel 2C). No significant differences were found in number of injuries or hemorrhages between wild and farmed fish, F1 and F2 + F3 cod from different broodstocks, F1 cod exposed to 60 V and those exposed to 120 V, and F1 cod exposed to 120 V with and without an inductor (Table 2). No spinal dislocations were noticed on the X-ray images taken from the other two cod showing paravertebral hemorrhages (Figure 3, panel 3C). Furthermore, X-ray examination revealed chronic malformations of the vertebral column in cod from all groups. This consisted of two adjacent compressed vertebrae in 5% and 20% of the cod from groups W and F1, respectively. In approximately 60% of the cod from groups F2 and F3, either chronic compression of three or more adjacent vertebrae, ankylosis, lordosis, or chronic dislocation of more than one neural and hemal arch were observed. The mean number of vertebral bodies observed in the cod in this study is presented in Table 3.

Necropsy of the animals without spinal injuries revealed no macroscopic external or internal acute lesions. Table 3 presents the differences in the mean results for length, total and somatic weight, gonadosomatic index, intestine somatic index, Fulton's condition factor, and muscle factor. Inspection of the gills of F2 Atlantic Cod revealed multifocal white discoloration in 25% of the animals. Examination of the internal organs revealed nematodes in the livers of wild cod, while multifocal granulomas were often present in the liver, spleen, and kidney. In all other animals, no macroscopic abnormalities were found. Histological examination did not reveal any acute lesions. Melanomacrophage aggregates were observed in all spleen and kidney samples and half of the liver samples of W cod. These aggregates were present in only 4% of F1 fish and 30% of F2 and F3 fish. They were often located in the kidney and sometimes in the spleen as well. Incidental findings included multifocal mild gill hyperplasia with intralesional protozoa in F2 cod, nematodes in the livers of wild cod, and multifocal granulomas in the livers, spleens, and kidneys of wild cod.
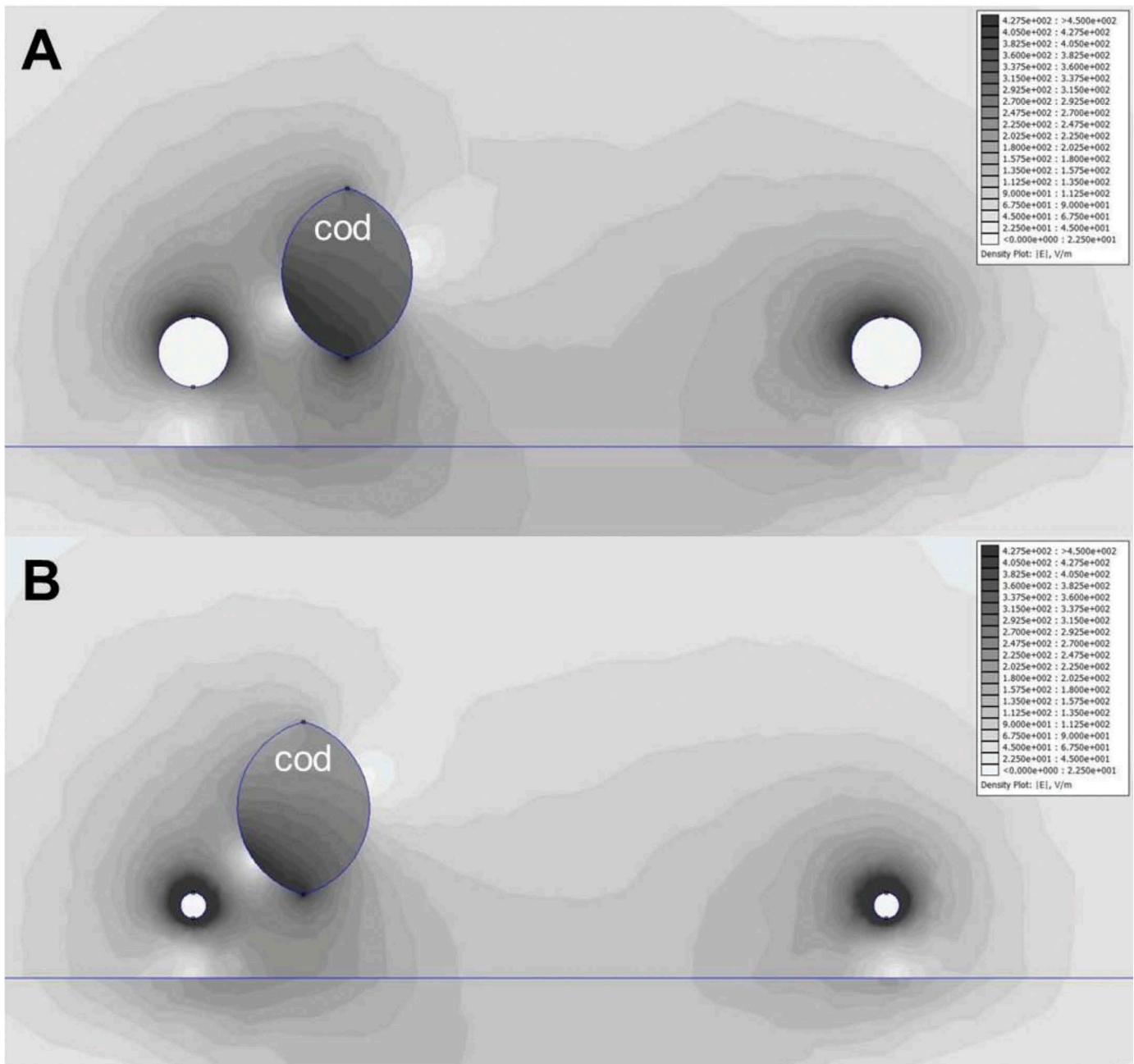
FIGURE 2. Qualitative approaching simulation of a cross section of an Atlantic Cod exposed to **(A)** the Delmeco electrodes (diameter, 26 mm) and **(B)** the Marelec electrodes (diameter, 12 mm) with a 60 V difference and a mutual distance of 0.325 m. Lighter gray represents the lower field strengths (V/m) present inside cod exposed to the thinner Marelec electrodes.

The mineral ratio is summarized in Table 3. After samples were thawed, the vertebrae of the F1 fish were more loosely connected and much easier to disconnect during processing both before and after boiling. In all groups, the vertebrae of larger animals also seemed to be slightly more tightly connected than those of small individuals.

DISCUSSION

Observed Effects

Behavioral reaction.—All Atlantic Cod exhibited a cramp reaction during exposure. This was expected, as the threshold frequency for inducing a cramp reaction in vertebrate species is reported to be between 20 and 30 Hz (Snyder 2003; Soetaert et al.

TABLE 2. *P*-values from the statistical comparison (Fisher's exact tests) of the epileptiform seizures, paravertebral hemorrhages, and spinal injuries in the Atlantic Cod listed in Table 1.

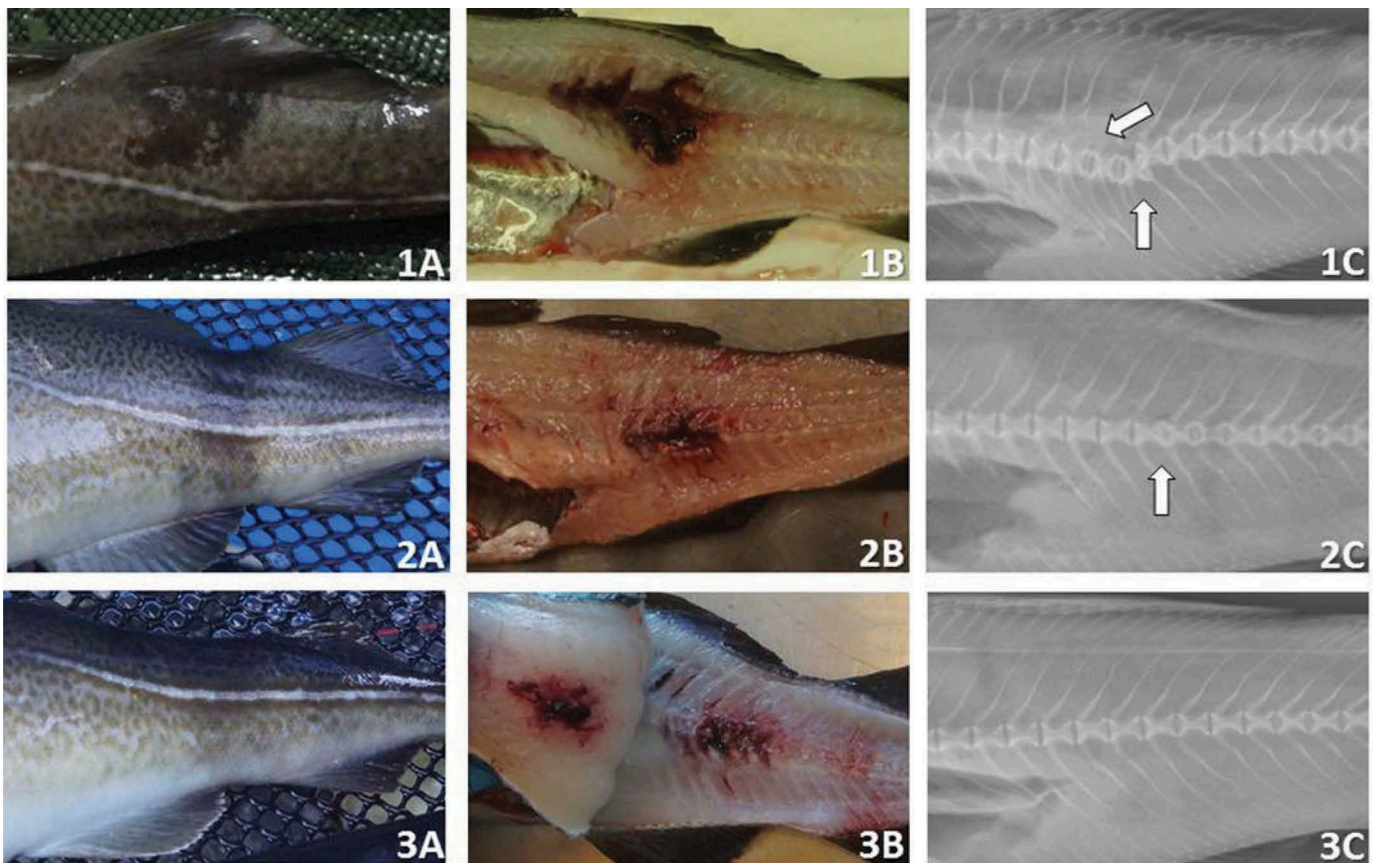| Statistical comparison	Pulse IDs	Epi	Hem	Inj
Identical exposed wild versus farmed cod	W(I) versus F1(I) + F2a(I) + F2b(I) + F3(I)	1	0.25	0.25
Farmed cod of different broodstocks (same pulse)	F1(I+II+III) versus F2a(I) + F2b(I) + F3(I)	1	0.56	1
F1 cod exposed to 60 versus 120 V	F1(I+II+III) versus F1(IV+V)	<0.001	0.07	0.08
F1 cod exposed to 120 V with versus without inductor	F1(IV) versus F1(V)	0.28	0.23	0.48

FIGURE 3. Imagess of (1) wild Atlantic Cod showing spinal luxation, (2) farmed F1 cod with spinal subluxation, and (3) affected F1 cod without spinal injury. The panels labeled A show the dark discoloration immediately after exposure, those labeled B the paravertebral hemorrhages noted at 14 d (1B) or 2 h (2B, 3B) postexposure, and those labeled C X-rays of an acute luxation with associated bone fractures (1C), an acute subluxation (2C), and an undamaged spine (3C).

2016), which is well below the frequencies applied in the present experiments. However, this cramp reaction was weaker in cod exposed near the thinner Marelec electrodes (diameter, 12 mm; pulse IDs IX and X) than near the thicker Delmeco electrodes (diameter, 26 mm). This may be explained by larger electrodes' having less electric resistance in water, resulting in larger radiated electric fields (Novotny 1990). If the diameter of the electrode is doubled, the current density at every point external to the electrodes will also be twice as large if the same potential difference is applied. This phenomenon is illustrated in the FEMM simulation, in which there were lower field strengths inside the fish's body part when it was lying near the thinner electrode. Nevertheless, it is remarkable that this surpasses the impact of only a small part of the cod's body being exposed effectively. Indeed, the 0.18-m-long Delmeco electrodes covered only the most cranial part of the 45-cm fish, whereas the entire

TABLE 3. Means (SDs) of physiological factors among the different groups of Atlantic Cod.

Factor	W	F1	F2a	F2b	F3
Sample size	25	80	27	35	38
Total length (cm)	39.0 (5.4)	40.1 (2.2)	68.6 (3.8)	72.5 (4.1)	45.28 (2.5)
Weight (kg)	0.6 (0.3)	0.8 (0.2)	4.0 (0.8)	4.3 (0.7)	1.0 (0.2)
Somatic weight (%)	91.3 (1.4)	81.8 (1.0)	69.4 (10.7)	79.3 (6.7)	85.2 (3.4)
Condition factor	0.82 (0.09)	1.04 (0.07)	0.85 (0.11)	0.90 (0.13)	0.94 (0.08)
Muscle factor	0.34 (0.06)	0.61 (0.06)	0.35 (0.05)	0.43 (0.08)	0.48 (0.06)
Gonadosomatic index (%)	0.4 (0.2)	0.5 (0.4)	25.0 (22.8)	2.2 (1.9)	3.2 (2.1)
Intestine somatic index (%)	9.1 (1.6)	21.3 (1.5)	19.7 (6.0)	20.6 (5.4)	14.2 (2.6)
Number of vertebrae	52.6 (0.7)	51.8 (0.7)	51.5 (0.5)	51.5 (0.5)	52.5 (1.0)
Vertebral mineral content (%)	64.3 (0.9)	61.8 (0.3)	60.7 (0.6)	61.0 (0.2)	60.4 (0.7)

body of the cod was exposed near the 1-m-long Marelec electrodes. This suggests that the stimulation of receptors located in the cranial part of the fish's body may be more important in determining the intensity of the muscle contraction than direct muscle stimulation.

The behavioral reaction of the Atlantic Cod immediately after exposure to the cramp pulse used by electrotrawls targeting Sole (60 V) was an escape response. However, when the amplitude was doubled to 120 V—and, as a consequence, the power almost quadrupled—epileptiform seizures occurred in 11 of 20 individuals. Such epileptiform seizures result from overstimulation of the brain, as may be inferred from the increased number of seizures when the cranial region was exposed to the highest field strength. Indeed, next to frequency, field strength has been reported to be the predominant factor in generating epileptiform seizures (Sharber and Black 1999; Roth et al. 2004; Soetaert et al. 2016). Sharber et al. (1994) stated that the myoclonic jerks associated with these epileptiform seizures induce spinal injuries. This was not confirmed by our data, since none of the 11 cod showing epileptiform seizures were injured whereas 3 of the 9 animals that remained fully conscious during exposure displayed paravertebral hemorrhages. Moreover, in previous studies reporting spinal injuries when adult cod were exposed to commercial pulse settings up to 60 V, epileptiform seizures were never observed (De Haan et al. 2011).

Physical injuries.—Acute spinal injuries are often associated with dark discoloration of the integument, which is believed to be caused by dilatation of skin melanophores, possibly as a result of sympathetic nerve damage and stimulation (Sharber and Black 1999). In the present study, spinal injuries diagnosed by means of radiographic imaging were always associated with hemorrhages in the musculature flanking the vertebral column. However, no spinal injuries were encountered in two of the five animals displaying paravertebral hemorrhages. This suggests that a temporary dislocation of vertebral bodies had occurred, possibly eliciting a tear in the caudal vessels situated in the hemal canal, after which the vertebrae resumed their normal position. Besides,

the site of the spinal injuries was an intriguing finding. They were always located between the 20th and 25th vertebrae and ventral to the caudal part of the second dorsal fin, as has been reported previously (De Haan et al. 2011; van Marlen et al. 2014; Soetaert et al. 2016). This is the transitional zone between the abdominal and tail regions, where the compression force imposed by the lateral muscle during swimming is presumed to be strongest. The same explanation has also been advanced for the finding that the 23rd vertebral body is also most frequently affected by lordosis in farmed Atlantic Cod (Opstad et al. 2013). In other species the location of spinal injuries varies (Snyder 2003) but is believed to be in regions with the strongest muscle contractions (Dolan and Miranda 2004). Remarkably, the part of the body in which the spinal injuries were located was not situated between the conductors and thus was barely stimulated electrically. Hence, it is unlikely that these spinal injuries were caused by direct muscle stimulation or stimulation through the sensory nerves, as suggested by McBary (1956). The present data point to a reflex response after stimulation of the cranial sensory nerves and/or the brain. This hypothesis would also explain the weaker contractions of cod when the entire body was exposed near the Marelec electrodes. Indeed, the weaker behavioral reaction to the Marelec electrodes may result from the lower field strengths around the animal's cranial body part, regardless of the much higher field strengths surrounding the caudal part of the body. In addition, direct stimulation of the muscles would probably result in asymmetric cramps as the fish's side nearest to the conductor is stimulated more intensely. These elements might mean that stimulation of the cranial sensory nerves and/or brain is crucial to the intensity of the reactions noted. More research is needed to test this hypothesis.

Influence of Electric Settings and Setup

The results in Table 1 show that the electrical output of the laboratory pulse generator (pulse ID I) was similar to that of the Delmeco generator (pulse ID II) used in commercial fishing and in the study by De Haan et al. (2011). The latter research group applied the following pulse settings: 80-Hz

PBC, 57 Vp, 68 Ap, 254-µs pulse duration, and 220-µs rise time. In the present study, an identical experimental setup (identical exposure cage, electrode pair, electrode distance, and powerleads) and almost identical pulse parameter settings (80-Hz PBC, 60 Vp, 72–82 Ap, 270–310-µs pulse duration, and 40–220-µs rise time) were used for all experiments (pulse IDs I, II, III, VI, VII, and VIII) when the commercial settings were simulated. However, spinal injuries were observed in only 0–5% of the fish (pulse IDs I and II; Table 1), which is far less than the 50–70% observed by De Haan et al. (2011). The different injury rates in the two studies were obtained under similar experimental conditions, which indicates that they do not result from experimental artifacts. When the pulse amplitude and power were doubled and quadrupled, respectively, the injury rate of F1 cod increased to 0–30%. This inverse correlation between the occurrence of spinal injuries and/or paravertebral hemorrhages and pulse amplitude was previously observed by several authors (Snyder 2003; Schreer et al. 2004; De Haan et al. 2016). Nevertheless, this injury rate is still much lower than the 50–70% reported by De Haan et al. (2016).

Several other settings were altered to mimic the variability that occurs in commercial fishing. First, temperatures ranging from 3.5°C to 15°C were tested, including the temperature (7.5°C) at which De Haan et al. (2016) obtained 50–70% injuries, but no significant impact was observed. Second, the Delmeco electrodes were compared with the thinner Marelec electrodes. Although the Marelec electrodes are currently only used in electrotrawls targeting shrimp, they have recently been experimentally tested in electrified benthos release panels. When a cramp stimulus for Sole was applied, ±8% of the Atlantic Cod were found to have spinal injuries (Soetaert 2015). This indicates that even the weaker contractions in the proximity of the Marelec electrodes can cause significant injury rates, exceeding those observed in the present study. Third, the differences between treatments with and without an inductor (pulse IDs IV versus V) suggest that the rise time of the pulse may affect behavioral reactions and the injury rate, although no significant effect was found. As a consequence, electrotrawls using the HFK system (which has a short rise time) may have a different effect on cod than those using the Delmeco system (which has a longer rise time), although the effect is still unclear. Indeed, all F1 fish with spinal injuries were exposed to long rise times, whereas freshwater electrofishing research has reported much higher injury and mortality rates upon adopting exponential and quarter-sinus pulse shapes with short rise times (Halsband 1967; Lamarque 1967a,b, 1990; Vibert 1967). Last, the pulse type was switched from PBC to PAC (pulse IDs VI and X) and the orientation of the animal was switched to perpendicular (pulse ID VII), but no increase in injuries or changes in behavior were observed; this may be a result of the low number of animals included and/or the low numbers of injuries observed.

De Haan et al. (2011) reported that Atlantic Cod exposed 15 cm or more from the electrodes did not show spinal injuries but that up to 70% of the animals were harmed when exposed closer to the conductor. These findings accord with the 8–11% injury rates observed in the field (van Marlen et al. 2014; Soetaert 2015) with cod exposed at random points relative to the electrodes. However, only 0–5% of the cod exposed under very similar to identical conditions near the electrodes were observed to have spinal injuries in this study. Although pulse parameters such as the applied potential difference and electrode diameter could affect this injury rate, they cannot explain the differences with previous studies. It can therefore be concluded that the large variability in the occurrence of spinal injuries is not solely determined by the electric pulse parameters but that intrinsic fish properties may play a crucial role and hence need to be taken into account in studies of the impact of electric fishing.

Fish-Related Parameters

Size and condition.—Since the pulse and electrode settings were identical to those used by De Haan et al. (2011) but resulted in fewer lesions, fish-related parameters may have a much larger influence on the occurrence of spinal injuries in Atlantic Cod than previously assumed. Unfortunately, all of the fish groups included in our study showed very low injury rates and no significant differences in the occurrence of hemorrhages or spinal injuries were found. Nevertheless, important morphological variability was observed between the different groups, which may be the first indication of other decisive parameters and suggests detailed comparisons in future experiments.

Atlantic Cod exhibit seasonal dynamics in condition and muscularity (Schwalme and Chouinard 1999), which may affect fish's reactions to and muscle contractions induced by electric pulses. Therefore, physiological parameters were monitored based on the muscle factor and the Fulton's condition factor. The latter is linearly related to the muscle protein content in wild cod, which acts as an indicator of muscle quality and energy reserves (Lambert and Dutil 1997a). Both this condition factor and somatic weight show seasonal differences that are distinguishable in the results for the farmed fish: April (F2a: $K = 0.82$), May (F3: $K = 0.94$), June (F2b: $K = 0.90$), and October (F1: $K = 1.04$). Wild cod had a K-value of 0.82, which is close to the limit of 0.85 observed in well-fed wild cod (Lambert and Dutil 1997b). These K-values seem to be proportional to muscularity (expressed as the M-factor), as they were up to 79% higher in farmed cod than in wild cod (Table 3). As mentioned above, muscular contractions are probably the main cause of spinal injuries. Therefore, it is tempting to speculate that increased muscularity will result in a higher incidence of spinal injuries after electric exposure. However, no difference in injuries (Table 1) was observed between the weakened F2a fish with a low M-factor and F2b fish with a higher M-factor (Table 3). Additionally, F1 fish

with much greater muscularity than wild fish showed only a 3% injury rate, which is much lower than that observed in the field by van Marlen et al. (2014). Although muscularity can play a role, these findings demonstrate that other parameters need to be included as well.

The size of the animal can also be a determining factor. It is generally accepted that a larger fish, which has a larger potential difference over its body, will react more intensively to electric pulses (Emery 1984; Dalbey et al. 1996; Dolan and Miranda 2003). Indeed, in studies by De Haan et al. (2011), small Atlantic Cod (12–16 cm) exposed to electric pulses did not develop lesions. Dalbey et al. (1996) also found a positive correlation between the number and severity of the injuries and the size of Rainbow Trout *Oncorhynchus mykiss*, with larger animals showing more fractures and fewer vertebral compressions after electric exposure. In contrast, De Haan et al. (2016) observed a decrease in the probability of fractures with body size in adult cod, although no injuries were observed in juvenile cod (12–16 cm). In the present study, no fractures were observed in the largest cod of the F2 group. These observations make it tempting to speculate that small animals are also susceptible. This is in line with the results obtained by van Marlen et al. (2014), who described paravertebral hemorrhages in wild cod of 20–27 cm. However, as each fish group had a different origin and hence rearing history, other (co)decisive factors in terms of sensitivity to electric pulses cannot be excluded at this stage.

Vertebral characteristics.—Rearing conditions affect the phenotype and morphology of fish. Differences in rearing conditions can influence the phenotype (Galloway et al. 1998, 1999; Johnston et al. 1998), the number of vertebrae (Blaxter 1969; Brander 1979; Lear and Wells 1984), and mineralization (Kousoulaki et al. 2010) as well as deformities of the spinal column (Fjelldal et al. 2007, 2009, 2013). Important parameters that affect the spinal column are water quality and temperature as well as feed and nutrition. Since the different groups of Atlantic Cod in our study had different origins, the possible effects of rearing, muscularity, mineralization, and skeletal deformities were compared.

Differences in the number of vertebrae and muscle mass were previously suggested as possible reasons for the differences in vertebral injuries observed between salmonids and centrarchids in freshwater and Atlantic Cod and European Seabass in seawater (Soetaert 2015; Soetaert et al. 2015). The number of vertebrae varies between specific natural populations (Swain et al. 2001) and can be influenced by environmental factors. Blaxter (1969) stated that the water temperature, salinity, and oxygen level during early life stages may affect the number of vertebrae in teleosts. In addition, Brander (1979) reported an inverse relationship between water temperature and vertebral number during early development in cod. Despite their different rearing history, the cod in all our experimental groups had 52 ± 2 vertebrae (Table 3) and no aberrant numbers were found in the affected cod (Table 4).

Moreover, similar values are reported by De Haan et al. (2016) and described in the literature on cultured and wild cod (Fjelldal et al. 2013). It is therefore unlikely that the number of vertebrae was responsible for the observed variability between the studies.

The mechanical strength of vertebral bodies depends on their mineralization (Fjelldal et al. 2006), while the degree of mineralization depends on diet. As evidence of this, increased physiological bone resorption has been observed following exposure to phosphorus-deficient diets (Kousoulaki et al. 2010) as well as in migratory teleosts during fasting or sexual maturation (Sbaihi et al. 2009) and in fish experiencing increased levels of cortisol as a result of chronic stress (Sbaihi et al. 2009). Therefore, in our experimental setup we included wild Atlantic Cod maintained in indoor facilities for several months as well as farmed cod at different stages of gonadal development. The hypothesis of Sbaihi et al. (2009) was not confirmed in our study: the mineral content of gravid farmed fish (F2a) was similar to that of large juveniles from the same stock (F3). Furthermore, chronic skeletal deformities were encountered on radiography. In cultured cod, this is often related to a high growth rate during juvenile stages resulting in an increased incidence of spinal malformations of up to 75% (Fjelldal et al. 2009). It is possible that such chronic lesions alter the mechanical strength of the vertebral column, e.g., by making the caudal vertebral zone more rigid. This hypothesis might explain the resilience of F2 and F3 cod against spinal injuries, but much more data on injured fish will be necessary to test this hypothesis.

The mechanical strength of the vertebral column is also determined by the strength of the intervertebral ligaments. A weaker intervertebral connection may directly promote the occurrence of (temporary) luxations. It is therefore important to stress that the vertebrae of the F1 fish were more loosely connected than those of the F2 and F3 fish, as this may be a reason why the former were more prone to spinal injuries. Further, the strength and/or elasticity of the intervertebral ligaments may determine their capability to adhere to vertebrae or return them to their original position. This could explain why some paravertebral hemorrhages could not be associated with spinal injuries based on X-ray examination: the intervertebral ligaments may have returned the vertebrae to their normal position after an acute, electric-induced luxation. Further research should therefore include the biomechanical properties of the spinal column and assess how it is affected by rearing conditions.

None of the previously discussed breeding parameters had a conclusive effect on injuries. The factor(s) that explain the large difference in spinal injury rates between the Atlantic Cod exposed by De Haan et al. (2011) and the F1 cod in the present study (which were raised at the same farm and exposed in identical fashion and at the same time of the year) have yet to be discovered. The rearing history of the animals might set us on the road to elucidating the

TABLE 4. Means (SDs) of physiological factors among Atlantic Cod (1) exposed to different electrical settings (I, II, or V) but unaffected and (2) showing paravertebral hemorrhages as a result of exposure.

	Wild cod		Farmed cod (F1)		
Factor	Unaffected	Affected (I)	Unaffected	Affected (II)	Affected (V)
Number of cod	19	1	66	1	3
Total length (cm)	39.2 (5.8)	42.0	39.9 (2.6)	42.0	38.7 (1.2)
Weight (kg)	0.6 (0.3)	0.6	0.8 (0.2)	1.0	0.7 (0.0)
Somatic weight (%)	91.0 (1.3)	94.0	81.9 (1.8)	80.8	82.4 (1.1)
Condition factor	0.82 (0.10)	0.90	1.04 (0.08)	1.05	1.04 (0.04)
Muscle factor	0.33 (0.07)	0.41	0.61 (0.07)	0.66	0.62 (0.02)
Gonadosomatic index (%)	0.5 (0.2)	0.3	0.5 (0.4)	0.5	0.2 (0.1)
Intestine somatic index (%)	9.4 (1.6)	6.4	20.9 (2.5)	22.8	21.0 (1.8)
Number of vertebrae	52.7 (0.7)	54	51.9 (0.6)	52.0	51.3 (0.5)

determining parameter(s). The larval life stages of the cod used by De Haan et al. (2011) were reared extensively in a lagoon in which the temperature reached 20°C in summer, and they were fed zooplankton as described by Blom et al. (1991). In contrast, all of the farmed cod used in the present study were reared in closed tanks and fed rotifers and *Artemia* (Hansen et al. 2014). Both temperature and the feed are known to have an influence on a fish's development and growing speed (Galloway et al. 1998, 1999) and on the phenotype of numerous characteristics critical to swimming (Johnston et al. 1998). Faster growing speeds might for example affect intervertebral connections, making fish more prone to luxations. Although our key question remains unanswered, i.e., which parameter is decisive for the variable number of lesions in cod, the data obtained in this study offer new and intriguing perspectives for further research.

Conclusion

The reaction of Atlantic Cod exposed to commercial, wire-shaped electrodes (60 V) was less intense if electrodes with a smaller diameter were used. When the potential difference was raised to 120 V, a significant number of cod experienced epileptiform seizures. The present results demonstrate that the sensitivity of cod to electric-induced spinal injuries can vary between 0% and 70% under (almost) identical exposures near the electrode (60 V). This indicates that these injuries are determined not only by the pulse parameter settings but also by other factors. Although the absence of a sensitive group of cod did not enable determination of the decisive parameter(s), the effects of intervertebral ligaments and rearing conditions during early life merit attention in future research.

REFERENCES

Bagenal, T. B. 1978. Aspects of fish fecundity. Pages 75–101 *in* S. D. Gerking, editor. Ecology of freshwater fish production. Blackwell Scientific Publications, Oxford, UK.

Blaxter, J. H. S. 1969. Experimental rearing of pilchard larvae, *Sardina pilchardus*. Journal of the Marine Biological Association of the United Kingdom 49:557–575.

Blom, G., H. Ottera, T. Svasand, T. S. Kristiansen, and B. Serigstad. 1991. The relationship between feeding conditions and production of cod fry (*Gadus morhua* L.) in a semi-enclosed marine ecosystem in western Norway, illustrated by use of a consumption model. Ecology and Management Aspects of Extensive Mariculture 192:176–189.

Brander, K. 1979. Relationship between vertebral number and water temperature in cod. Journal du Conseil International pour l'Exploration de la Mer 38:286–292.

Dalbey, S. R., T. E. McMahon, and W. A. Fredenberg. 1996. Effect of electrofishing pulse shape and electrofishing-induced spinal injury on long-term growth and survival of wild Rainbow Trout. North American Journal of Fisheries Management 16:560–569.

De Haan, D., J. E. Fosseidengen, P. G. Fjelldal, and D. Burggraaf. 2011. The effect of electric pulse stimulation to juvenile cod and cod of commercial landing size. International Council for the Exploration of the Sea, Document C/141/11, Copenhagen.

De Haan, D., J. E. Fosseidengen, P. G. Fjelldal, D. Burggraaf, and A. D. Rijnsdorp. 2016. Pulse trawl fishing: characteristics of the electrical stimulation and the effect on behaviour and injuries of Atlantic Cod (*Gadus morhua*). ICES Journal of Marine Science 73:1557–1569.

Depestele, J., I. Ana, K. Degrendele, M. Esmaeili, H. Polet, M. Roche, K. Summerbell, L. R. Teal, B. Vanelslander, and F. G. O'Neill. 2016. Measuring and assessing the physical impact of beam trawling. ICES Journal of Marine Science 73(Supplement 1):i15–i26.

Dolan, C. R., and L. E. Miranda. 2003. Immobilization thresholds of electrofishing relative to fish size. Transactions of the American Fisheries Society 132:969–976.

Dolan, C. R., and L. E. Miranda. 2004. Injury and mortality of warmwater fishes immobilized by electrofishing. North American Journal of Fisheries Management 24:118–127.

Emery, L. 1984. The physiological effects of electrofishing. California–Nevada Wildlife Transactions 1984:59–62.

Fjelldal, G., T. J. Hansen, and A. E. Berg. 2007. A radiological study on the development of vertebral deformities in cultured Atlantic Salmon (*Salmo salar* L.). Aquaculture 273:721–728.

Fjelldal, P. G., E. J. Lock, S. Grotmol, G. K. Totland, U. Nordgarden, G. Flik, and T. Hansen. 2006. Impact of smolt production strategy on vertebral growth and mineralisation during smoltification and the early seawater phase in Atlantic Salmon (*Salmo salar* L.). Aquaculture 261:715–728.

Fjelldal, P. G., G. K. Totland, T. Hansen, H. Kryvi, X. Y. Wang, J. L. Sondergaard, and S. Grotmol. 2013. Regional changes in vertebra morphology during ontogeny reflect the life history of Atlantic Cod (*Gadus morhua* L.). Journal of Anatomy 222:615–624.

Fjelldal, P. G., T. van der Meeren, K. E. Jorstad, and T. J. Hansen. 2009. A radiological study on vertebral deformities in cultured and wild Atlantic Cod (*Gadus morhua* L.). Aquaculture 289:6–12.

Galloway, T. F., E. Kjorsvik, and H. Kryvi. 1998. Effect of temperature on viability and axial muscle development in embryos and yolk sac larvae of the northeast Arctic Cod (*Gadus morhua*). Marine Biology 132:559–567.

Galloway, T. F., E. Kjorsvik, and H. Kryvi. 1999. Muscle growth and development in Atlantic Cod larvae (*Gadus morhua* L.) related to different somatic growth rates. Journal of Experimental Biology 202:2111–2120.

Halsband, E. 1967. Basic principles of electric fishing. Pages 57–64 *in* R. Vibert, editor. Fishing with electricity: its application to biology and management. Fishing News Books, London.

Johnston, I. A., N. J. Cole, M. Abercromby, and V. L. A. Vieira. 1998. Embryonic temperature modulates muscle growth characteristics in larval and juvenile herring. Journal of Experimental Biology 201:623–646.

Kousoulaki, K., P. G. Fjelldal, A. Aksnes, and S. Albrektsen. 2010. Growth and tissue mineralisation of Atlantic Cod (*Gadus morhua*) fed soluble P and Ca salts in the diet. Aquaculture 309:181–192.

Lambert, Y., and J. D. Dutil. 1997a. Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of cod (*Gadus morhua*)? Canadian Journal of Fisheries and Aquatic Sciences 54:104–112.

Lambert, Y., and J. D. Dutil. 1997b. Condition and energy reserves of Atlantic Cod (*Gadus morhua*) during the collapse of the northern Gulf of St. Lawrence stock. Canadian Journal of Fisheries and Aquatic Sciences 54:2388–2400.

Lear, W. H., and R. Wells. 1984. Vertebral averages of juvenile cod, *Gadus morhua*, from coastal waters of eastern Newfoundland and Labrador as indicators of stock origin. Journal of Northwest Atlantic Fishery Science 5:23–31.

McBary, B. M. 1956. The effect of electric fields on marine fishes. Marine Research 1:1–32.

Meeker, D. C., Finite element method magnetics, version 4.0.1. Available: http://www.femm.info. (July 2016).

Miranda, L. E., and C. R. Dolan. 2003. Test of a power transfer model for standardized electrofishing. Transactions of the American Fisheries Society 132:1179–1185.

Novotny, D. W. 1990. Electrical fishing apparatus and electric fields. Pages 34–88 *in* I. G. C. P. Lamarque, editor. Fishing with electricity. Blackwell Scientific Publications, Oxford, UK.

Opstad, I., P. G. Fjelldal, O. Karlsen, A. Thorsen, T. J. Hansen, and G. L. Taranger. 2013. The effect of triploidization of Atlantic Cod (*Gadus morhua* L.) on survival, growth, and deformities during early life stages. Aquaculture 388:54–59.

Righton, D., J. Metcalfe, and P. Connolly. 2001. Fisheries: different behaviour of North and Irish Sea cod. Nature 411:156.

Roth, B., D. Moeller, and E. Slinde. 2004. Ability of electric field strength, frequency, and current duration to stun farmed Atlantic Salmon and Pollock and relations to observed injuries using sinusoidal and square wave alternating current. North American Journal of Aquaculture 66:208–216.

Sbaihi, M., A. Kacem, S. Aroua, S. Baloche, K. Rousseau, E. Lopez, F. Meunier, and S. Dufour. 2007. Thyroid hormone–induced demineralisation of the vertebral skeleton of the eel, *Anguilla anguilla*. General and Comparative Endocrinology 151:98–107.

Sbaihi, M., K. Rousseau, S. Baloche, F. Meunier, M. Fouchereau-Peron, and S. Dufour. 2009. Cortisol mobilizes mineral stores from vertebral skeleton in the European Eel: an ancestral origin for glucocorticoid-induced osteoporosis? Journal of Endocrinology 201:241–252.

Schwalme, K., and G. A. Chouinard. 1999. Seasonal dynamics in feeding, organ weights, and reproductive maturation of Atlantic Cod (*Gadus morhua*) in the southern Gulf of St. Lawrence. ICES Journal of Marine Science 56:303–319.

Sharber, N. G., and J. S. Black. 1999. Epilepsy as a unifying principle in electrofishing theory: a proposal. Transactions of the American Fisheries Society 128:666–671.

Sharber, N. G., S. W. Carothers, J. P. Sharber, J. C. de Vos, and D. A. House. 1994. Reducing electrofishing-induced injury of Rainbow Trout. North American Journal of Fisheries Management 14:340–346.

Snyder, D. E. 2003. Invited overview: conclusions from a review of electrofishing and its harmful effects on fish. Reviews in Fish Biology and Fisheries 13:445–453.

Soetaert, M. 2015. Electrofishing: exploring the safety range of electric pulses for marine species and its potential for further innovation. Doctoral dissertation. Ghent University, Ghent, Belgium.

Soetaert, M., K. Chiers, L. Duchateau, H. Polet, B. Verschueren, and A. Decostere. 2014. Determining the safety range of electrical pulses for two benthic invertebrates: brown shrimp (*Crangon crangon* L.) and ragworm (*Alitta virens* S.). ICES Journal of Marine Science 72:973–980.

Soetaert, M., A. Decostere, H. Polet, B. Verschueren, and K. Chiers. 2015. Electrotrawling: a promising alternative fishing technique warranting further exploration. Fish and Fisheries 16:104–124.

Soetaert, M., A. Decostere, B. Verschueren, J. Saunders, A. Van Caelenberge, V. Puvanendran, A. Mortensen, L. Duchateau, H. Polet, and K. Chiers. 2016. Side effects of electrotrawling: exploring the safe operating space for Dover sole (*Solea solea* L.) and Atlantic Cod (*Gadus morhua* L.). Fisheries Research 177:93–103.

Swain, D. P., K. T. Frank, and G. Maillet. 2001. Delineating stocks of Atlantic Cod (*Gadus morhua*) in the Gulf of St. Lawrence and Cabot Strait areas using vertebral number. ICES Journal of Marine Science 58:253–269.

Van Marlen, B., J. A. M. Wiegerinck, E. van Os-Koomen, and E. van Barneveld. 2014. Catch comparison of flatfish pulse trawls and a tickler chain beam trawl. Fisheries Research 151:57–69.

Vibert, R. 1967. General report of the working party on the applications of electricity to inland fishery biology and management. Fishing News Books, London.