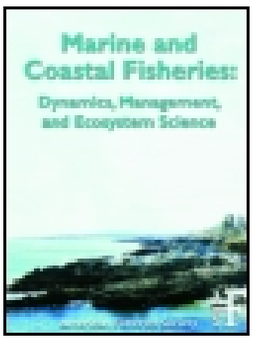

Taylor & Francis
Taylor & Francis Group

Marine and Coastal Fisheries

Dynamics, Management, and Ecosystem Science

ISSN: (Print) 1942-5120 (Online) Journal homepage: http://www.tandfonline.com/loi/umcf20

Impact of Pulsed Direct Current on Embryos, Larvae and Young Juveniles Atlantic cod (Gadus morhua) and its Implication on Electrotrawling for Brown Shrimp

Desender Marieke, Decostere Annemie, Adriaens Dominique, Duchateau Luc, Mortensen Atle, Polet Hans, Puvanendran Velmurugu, Verschueren Bart & Chiers Koen

To cite this article: Desender Marieke, Decostere Annemie, Adriaens Dominique, Duchateau Luc, Mortensen Atle, Polet Hans, Puvanendran Velmurugu, Verschueren Bart & Chiers Koen (2017): Impact of Pulsed Direct Current on Embryos, Larvae and Young Juveniles Atlantic cod (Gadus morhua) and its Implication on Electrotrawling for Brown Shrimp, Marine and Coastal Fisheries, DOI: 10.1080/19425120.2017.1321592

To link to this article: http://dx.doi.org/10.1080/19425120.2017.1321592

Accepted author version posted online: 24 Apr 2017.

Impact of pulsed direct current on embryos, larvae and young juveniles Atlantic cod (*Gadus morhua*) and its implication on electrotrawling for brown shrimp.

Desender Marieke[1,2*], Decostere Annemie[4], Adriaens Dominique[3], Duchateau Luc[5], Mortensen Atle[6], Polet Hans[2], Puvanendran Velmurugu[6], Verschueren Bart[2], Chiers Koen[4]

[1] Department of Morphology, Faculty of Veterinary Medicine – Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

E-mail: marieke.desender@ugent.be

[2] Institute for Agricultural and Fisheries Research (ILVO), Animal Sciences - Fisheries, Ankerstraat 1, 8400 Oostende, Belgium

[3] Research Group Evolutionary Morphology of Vertebrates, Department of Biology, Faculty of Science – Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium

[4] Department of Pathology, Bacteriology & Poultry Diseases, Faculty of Veterinary Medicine – Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

[5] Department of Comparative physiology and biometrics, Faculty of Veterinary Medicine – Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

[6] The Norwegian Institute of Food, Fisheries and Aquaculture Research (NOFIMA), Muninbakken 9-13, Breivika P.O. Box 6122, NO-9291 Tromsø, Norway

*** Corresponding author:** Marieke Desender; E-mail: *marieke.desender@ugent.be*; Tel.: +32 9 264 77 14

Abstract

The application of electrical pulses in fishing gear is considered a promising option to increase the sustainability of demersal trawl fisheries. In the electrotrawl fishery for brown shrimp, an electrical field selectively induces a startle response in shrimp. Other benthic organisms remain mainly on the seafloor and escape underneath a hovering trawl. Previous experiments indicated that this pulse has no short-term major harmful effect on adult fish and invertebrates. However, the impact on young marine life stages is still unknown. As brown shrimp are caught in shallow coastal zones and estuaries, important nurseries or spawning areas for a wide range of marine species, electrotrawling on these grounds could therefore harm embryos, larvae and juveniles.

In this study, experiments were carried out on different developmental stages of cod (*Gadus morhua*) which are considered vulnerable to electrical pulses. Three embryonic, four larval and one juvenile stage were exposed to a homogeneous electrical field of 150 V/m_{peak} for 5 s mimicking a worst case scenario. No significant differences in embryo mortality rate were found between control and exposed groups. However, in the embryonic stage exposed at 18 days post fertilization (DPF), the initial hatching rate was lower. Larvae exposed at 2 and 26 days post hatching (DPH), exhibited a higher mortality rate than the corresponding non-exposed groups. In the other larval and juvenile stages, no short-term impact of exposure on survival was observed. Morphometric analysis of larvae and juveniles revealed no differences in measurements or deformations of the yolk, notochord, eye and head. Although exposure to a worst case electrical field did not impact the survival or development of six out of eight young life stages of cod, the observed delayed hatching rate and decreased survival for larvae might indicate an impact of electric pulses and warrants further research.

Keywords: Pulse fishing, life stages, survival, development, cod, morphometrics

Introduction

Beam trawls are used extensively in the North Sea to catch brown shrimp (*Crangon crangon* L.) and flatfish, in particular sole (*Solea solea* L.) and plaice (*Pleuronectes platessa* L.) (STECF, 2014). However, this demersal fishing technique negatively impacts the marine environment with high by-catch rates, intense bottom contact and high fuel consumption as major drawbacks (Jennings and Kaiser, 1998; Lindeboom and de Groot, 1998; Paschen et al., 2000). In order to deal with the upcoming discard ban and contribute to a more ecosystem-based approach in fisheries management (FAO 2009; 2012), one should strive toward measures mitigating the disadvantages of traditional beam trawling by reducing seabed contact and enhancing selective fishing (Polet, 2002; Revill and Holst, 2004; Catchpole et al., 2008). Electrical pulse fishing offers a promising alternative technique meeting these requirements (Boonstra and De Groot, 1974; Polet et al., 2005a; Soetaert et al., 2015). Using these devices, the mechanical stimulation in the ground gear by tickler chains, chain matrices or bobbins is (partly) replaced by electrodes providing electric pulses .

The electrotrawl targeting brown shrimp uses a 5 Hz low-frequency pulsed direct current (PDC) of 0.5 ms creating a field strength of minimum 30 V/m between two thread-shaped electrodes placed in parallel at a distance of 60 cm. In this way, a startle response (tail-flip) is selectively induced in the shrimp, forcing them to rise into the water column (Polet et al., 2005a). Other benthic organisms remain mainly on the seafloor and can subsequently escape underneath an elevated groundrope (Polet et al., 2005b). Therefore in the original electrotrawl for brown shrimp, the so called Hovercran, all 36 bobbins attached to the ground rope of the conventional trawl, used to mechanically startle the shrimp and protect the ground gear, are removed (Verschueren and Polet, 2009; Verschueren et al., 2012). However, at sea, vessels differ in rigging, gear configuration and number of bobbins used, resulting in different outcomes regarding selectivity and bottom contact (Verschueren and Vanelslander, 2013; Verschueren et al., 2014). To exemplify this, a commercial electrotrawl for brown shrimp was monitored in the Dutch Wadden Sea in

2013. However, also 11 bobbins were implemented on a modified straight bobbin rope, instead of 36 bobbins and a traditional U-shaped bobbin rope used in a traditional gear. A 76% decrease in discard amount and a 60% reduction of seabed contact resulting in 23% less drag resistance were noted (Verschueren et al., 2014). Furthermore, the catch volume of commercial shrimp was increased in summer with 16%, especially in clear water with low turbidity and during daylight. For the above mentioned reasons, the use of electrical pulses in fishing gear is regarded as a promising fishing method both from an economic and environmental point of view.

Fishing with electricity in the sea has been prohibited since 1998 in Europe (EU, 1998). In 2009, an exemption was granted allowing each member state to equip 5% of its beam trawl fleet with electrical pulse gears in the southern part of the North Sea (EU, 2009). In 2013, 42 additional licences were allocated to Dutch fisheries (EU, 2013). In view of the rapid expansion of electrotrawling, there is an urgent need to improve our knowledge on possible adverse effects of these pulses (Yu et al., 2007; Quirijns et al., 2015). Introducing a fishing method based on this technology without a sound knowledge on the interactions between pulse fishing and both target and non-target marine organisms would violate principles of responsible fishing (FAO, 2011).

Previously, in short-term laboratory conditions, the electric fields used in the shrimp pulse fishery seemed to have a limited impact on exposed adults of targeted or bycaught organisms (Polet et al., 2005a; Desender et al., 2016, 2017; Soetaert et al., 2014). Still, the potential impact on young life stages is a growing concern that has not been addressed.

As electrofishing is a commonly used sampling technique in rivers, ponds and lakes, research on the impact of electrical currents on eggs, larvae and juveniles previously focused on freshwater species. Indeed, electrical fields could negatively affect young organisms, with intensity and type of electrical field, exposure duration, developmental stage and species as determining parameters (Snyder 2003). However, one should keep in mind that data resulting from the use of electrical currents applied in fresh water with different electrical settings cannot necessarily be extrapolated to the a marine environment due to differences in conductivity. Field intensity used

in freshwater is 2-6 times higher and duration 10-60 times longer than in seawater. As these are the most critical parameters affecting embryos and larvae (Dwyer et al., 1993; Dwyer and Erdahl, 1995), it might be assumed that effects would be more moderate in seawater than freshwater (Soetaert et al., 2015). Whether the latter hypothesis is correct needed to be empirically investigated. Indeed, studies addressing the effects of electrofishing on young life-stages of marine species are not available. These data nevertheless are crucial as brown shrimp are often caught in shallow coastal zones adjacent to extensive tidal flat areas such as the Wadden Sea that are often important nurseries and spawning areas for a wide range of marine species.

The current study is the first to expose Atlantic cod (*Gadus morhua* L.) at various embryonic, larval and young juvenile stages to electrical pulses targeting brown shrimp and to evaluate their survival. Exposure might not cause a significant increase in mortality but may reduce growth rates for at least a few weeks (Muth and Rupert, 1997). Therefore, morphometric analysis was performed at two chosen time points for each developmental stage. Atlantic cod was adopted as a model organism for marine cold water round fish species. This commercially important top predator is considered vulnerable to high-frequency electrical pulses as observed in commercial catches on board flatfish pulse trawlers (Rasenbergh et al., 2013; Van Marlen et al., 2014) and during laboratory experiments performed by de Haan et al. (2009; 2011; 2016).

Materials and methods

Experimental animals

Fertilized eggs from strip-spawned captive broodstock of three different spawning events, batches, maintained at the Centre for Marine Aquaculture Research (NOFIMA, Tromsø, Norway) were incubated until the desired developmental stage. When approximately 50% of hatching occurred, this was referred to as 0 DPH (days post hatching) in larval age. In total eight developmental stages were exposed, resulting in eight experiments, ranging from early cleavage in the embryonic stages (batch 1), to larval stages (batch 2) to juveniles following metamorphosis

(batch 3). An overview of the experiments are illustrated in Table 1. Three stages of embryos at 1, 5 and 18 DPF (days post fertilization) (experiment 1-3), four larval stages at 2, 11, 26 and 46 DPH (experiment 4-7) and one young juvenile stage at 60 DPH (experiment 8) were exposed to electrical pulses as described below. Each experiment was performed in triplicate. Three control groups were also included, of which the fish were treated in exactly the same way as the exposed animals except for the fact that the electrical field was not switched on.

After exposure, survival was counted until 2DPH every five days for exposed embryos, one week past exposure for exposed larvae and 29 days following exposure for juveniles. Also morphometric characteristics were measured (Figure 1). Samples for morphometric analysis were taken at two time points for each experiment. The first time point was at 2DPH for embryos and one day following exposure for larvae and juveniles. The second time point was at least 15 days and maximum 31 days following exposure.

All experiments were approved by the Norwegian animal experimental ethical committee (FOTS ID 5185).

Housing and rearing

All organisms were cultured according to protocols applied at the Centre for Marine Aquaculture Research (Hansen and Puvanendran, 2010; Hansen et al., 2015). The embryos (3200 L^{-1}) were housed in 25 L black cylindroconical tanks. Larvae (120 L^{-1}) were transferred at two DPH to green cylindrical tanks of 190 L supplied with aeration (Hansen et al., 2015). Seawater was provided to all tanks with a flow through system connected to the nearby fjörd (salinity, 32 ‰; pH, 8.1; oxygen, 9 mg L^{-1}; NH_3, <0.004 mg L^{-1}). Water temperature ranged between 4.0 - 4.5 °C for the embryos and it was gradually raised to 10 °C for larvae from 5 to 10 DPH. Two ml of algae (*Nannochloropsis*, Reed Mariculture, Campbell, CA, USA) per day were provided from 2 DPH until 12 DPH. From 2 DPH until 29 DPH and from 25 DPH until 55 DPH, rotifers and *Artemia franciscana* nauplii were delivered as live food, respectively. Prey densities (5-10 rotifers mL^{-1}; 1-10 *Artemia* mL^{-1}) were increased during rearing. At 38 DPH, larvae to be exposed as juveniles received 15 g

dry feed (AlgaNorse Extra, Trofi AS, Tromsø, Norway) each day. This amount was increased to 60 g at 57 DPH, while *Artemia* prey densities were decreased gradually before they were discontinued (Hansen et al., 2015). Dead embryos and larvae were removed daily and 2-3 times every week, respectively.

Exposure to electrical pulses

Before each exposure the number of embryos or larvae in the incubator tank was estimated by counting the organisms in a 50ml vial. In this way, the appropriate number of embryos (15,000) or larvae (3,200) was transferred from the incubator tank to a plastic 33 x 24.5 x 21 cm exposure chamber that contained 12 L of seawater (Figure 1). Within the chamber, two plate-shaped stainless steel electrodes (32 x 23 x 0.4 cm), conformed to the cross-sectional area of the chamber, were fixed in parallel at 24.5 cm apart and connected to the output of an adjustable laboratory pulse generator (LPG, EPLG bvba, Belgium) (Stewart 1972; Bohl et al., 2010; Henry and Grizzle 2004). The LPG generator was set to produce a unipolar square-wave pulsed direct current. Electrical output settings generated were 5 Hz frequency and 500 µs pulse duration resulting in a 0.25% duty cycle, similar to the pulse used to catch brown shrimp at sea (Verschueren and Polet 2009; Verschueren and Vanelslander, 2013). To create a homogeneous electrical field of approximately 150 V/m an intensity of 36 V_{peak} was applied. The embryos, larvae and young juveniles were exposed for 5 s while being orientated in random directions.

Experimental set up

Exposure of embryos

Approximately 15,000 embryos were exposed once at each stage 1, 5 or 18 days post fertilisation (DPF) (experiment 1, 2 and 3, respectively). Following exposure, each group of 15,000 embryos was transferred to a new cylindroconical 25 L incubator (600 L^{-1}). In total 3 exposure and 3 control tanks were occupied at each developmental stage. The embryo mortality rate was estimated by counting the number of viable embryos in triplicate 50 ml vials taken from each aerated tank every 5 days until 23 DPF (2 DPH) at which time all embryos hatched into larvae.

During the hatching process at 21 DPF (0 DPH), the hatching rate was examined by counting the proportion of hatched larvae and the number of viable organisms in three 50 mL vials per tank. Dead embryos were removed daily until 2 DPH. At 2 DPH the number of larvae per tank was estimated and standardized at 3,000 larvae in 25 L^{-1} (120 L^{-1}). After hatching, 20 larvae per tank were sampled on a weekly basis until 53 DPH for a morphometric analysis described below.

Exposure of larvae

Electrical pulses were given to 3,200 larvae at each stage 2, 11, 26 or 46 DPH (experiment 4, 5, 6 and 7, respectively). Following exposure, the animals were maintained in two subgroups of exactly 200 (8 L^{-1}) and approximately 3,000 larvae (120 L^{-1}) in two 25 L cylindroconical tanks. In total 12 tanks (6 exposure and 6 control tanks) were occupied at each developmental stage. At one week post exposure, the surviving larvae in the former subgroup of 200 larvae were counted and sacrificed. From the latter subgroup of 3000 larvae, 20 larvae were sampled every week until 58 DPH for morphometric analysis.

Exposure of juveniles

At 60 DPH (experiment 8), 200 juveniles were counted, exposed and maintained in a 190 L tank (1 L^{-1}). In total 3 control and 3 exposure tanks were occupied. On a weekly basis, 20 juveniles per tank were sampled for morphometric analysis and processed as described below. At 89 DPH, the surviving animals were counted and sacrificed.

Morphometric analysis

All sampled specimens were sacrificed with an overdose of 0.7 g L^{-1} MS 222 (Sigma-Aldrich, Oslo, Norway). The animals were then fixed in a 3% buffered glutaraldehyde solution and stored in 10 mL vials (Glauert, 1987) for morphometric analysis. For each developmental stage, the samples of two time points were processed for morphometric analysis as described below. The first time

point was 1 DPH for the embryonic stages (experiment 1-3) and one day post exposure for the larval and juvenile stages at 3, 12, 27, 47 and 61 DPH (experiment 4-8). For all exposed embryonic stages, the second time point was 22 DPH. Larval stages exposed on 2, 11, 26 and 47 DPH were subjected to a morphological analysis at 26, 27, 58 and 64 DPH, respectively (Table 1). The second sample point for juveniles was 89 DPH. Specimens were photographed with AnalySIS GetIT software with an Olympus Altra 20 digital camera mounted on an Olympus SZX9 microscope equipped with an x 0.5 planar lens (www.olympus.com). Larvae were placed horizontally in a 100 µL seawater droplet on glass slides, with their left and right palatoquadrate cartilages vertically aligned (Nikolakakis et al., 2014). The straight notochord length, from the rostral tip of the upper jaw to the caudal tip of the notochord; the total notochord length, measured in segments from the tip of the upper jaw along the notochord to its caudal end; eye diameter, vertical eye length; head height, through the middle of the eye perpendicular onto the notochord; and muscle height, vertical length of the notochord muscle near the posterior tip of the gut or anus, were measured using ImageJ v1.46 (Figure 2). The ratio of straight notochord length on total notochord length was calculated as a measurement of the curvature of larvae/juveniles. Additionally, for the larvae sampled until 3 DPH, the yolk surface was measured.

Statistics

According to the Shapiro-Wilk test, the embryo mortality rate was normally distributed. Therefore, the effect of the exposure on the embryo mortality rate was analysed by a mixed model. Replication was set as random effect and exposure, time and their interaction as categorical fixed effects. The analyses were done separately for each different exposure timing, i.e., at 1, 5 and 18 DPF. The effect of the exposure on the hatching rate at 0 DPH, and the mortality rate of larval and juvenile stages was analysed by a generalized mixed model with binomial error term (Stroup, 2012). Replication was set as random effect and developmental stage, exposure and their interaction as categorical fixed effects. The different length measurements were analysed by a mixed model with replicate as random effect and sample time,

developmental stage, treatment and interaction between developmental stage and treatment as fixed effects.

RESULTS

No significant differences in embryo mortality rate were found when exposure took place at 1 ($F_{1,32}$=0.04; P=0.837), 5 ($F_{1,26}$=0.84; P=0.369) or 18 ($F_{1,14}$=0.08; P=0.776) DPF (Figure 3). All groups started hatching on 20 DPF. Fifty percent hatching (0 DPH) occurred on 21 DPF. In the beginning of the hatching process, at 0 DPH, no significant differences in hatching rates were encountered between control and exposed groups for embryos exposed at 1 or 5 DPF, but a significant difference occurred in the embryos exposed at 18 DPF (OR=1.43, P=0.024), with a lower initial hatching rate in the exposed group (0.27, 95% CI:[0.23;0.32]) compared to the control group (0.35, 95% CI:[0.30;0.40]) (Figure 4). However, survival of larvae at 2DPH did not differ significantly from untreated controls ($F_{1,14}$=0.08; P=0.776).

In the trials investigating larval survival (Figure 5), survival differed significantly between the exposed and control groups when exposed at 2, 11 and 26 DPH, but not for later exposures at 46 and 60 DPH. At 2 DPH, a lower survival percentage (P=0.033) was observed in the exposed group (0.10, 95% CI:[0.08;0.11]) as compared to the control group (0.22, 95% CI:[0.10;0.40]). For 26 DPH exposure, this difference was even larger (P=0.001), with survival percentage in the exposed group equal to 0.53 (95% CI:[0.46;0.61]) and in the control group 0.69 (95% CI:[0.63;0.75]). At 11 DPH, a higher survival percentage (P=0.048) was observed in the exposed group (0.35, 95% CI:[0.25;0.46]) as compared to the control group (0.19, 95% CI:[0.11;0.31]).

No significant differences between control and exposed groups at any of the morphometric measurements (all P>0.527), nor for the incurvation ratio (P=0.166) were observed at sample points one or two.

DISCUSSION

Cod embryos were exposed during early-cleavage, epiboly and near hatching on 1, 5 and 18 DPF, respectively. No differences in embryo mortality rate between exposed cod embryos and controls were found at any of the three egg stages. Reported effects on early life stages, limited primarily to salmonids, are often contradictory (Snyder, 2003). Nevertheless, a sufficient number of studies indicate that electrofishing in freshwater over spawning grounds may harm embryos on, or in, the substrate. Survival was affected, particularly when exposure happened between pre-cleavage and eyed-egg stages (Godfrey, 1957; Lamarque, 1990). This early stage of development was also most vulnerable when exposed to mechanical shocks (Kolz and Reynolds, 1990; Dwyer et al., 1993). According to Rollefsen (1930) younger cod embryos in early-cleavage, are more susceptible towards external influences than embryos at later stages as they are only covered by a thin layer of protoplasm. After the completion of epiboly during gastrulation, the yolk is covered by a thin layer of embryonal tissue resulting in increased resistance to external influences. Mortality before epiboly is completed may therefore be caused by rupture of the vitelline membrane or the protoplasm layer of the yolk (Hayes 1949; Godfrey 1957). Breakdown of the cell membrane may also occur when pores created during electroporation fail to reseal (Chen et al., 2006). Epiboly has hence been identified as the most sensitive stage during development to these stressors in different species (Muth and Rupert, 1997; Roach, 1999; Henry and Grizzle, 2004).

In contrast, several hypotheses as to why electrical pulses did not elicit a negative impact on these vulnerable life stages may be advanced. Cod eggs are relatively small, 1.16 – 1.89 mm (Andersen et al., 1994; Auditore et al., 1994). As transmembrane potential increases with cell radius (Gaylor, 1988), several studies confirmed that electroshock-induced mortality increases with egg size (Henry and Grizzle, 2004; Bohl et al., 2010). Survival is known to decrease when voltage levels increase. A voltage gradient of 8-16 V/cm DC was needed to cause significant mortality in fresh water species such as largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*) with a comparable egg size of approximately 1.7 and 1.1 mm respectively (Henry &

Grizzle, 2004). In the present trials, a lower intensity of 1.5 V/cm was applied in seawater to the cod eggs to simulate the shrimp pulse. Also electrical current type may be critical to embryo survival. Pulsed direct current as used in our experiments has resulted in higher survival than direct currents (DC) (Dwyer and Erdahl, 1995; Keefe et al., 2000; Henry and Grizzle, 2004).

Electrical fields may induce premature hatching, as observed in bluegill and medaka (*Oryzias latipes*), resulting in an *in situ* increased risk of predation and consequently higher mortality (Yamagami, 1988; Henry & Grizzle, 2004). In the current study, no higher hatching rate at 0 DPH was observed in exposed groups. In contrast, a lower initial hatching rate was noted for eggs exposed at 18 DPF. The reason for this finding is not clear, but might be attributed to chemical reactions with seawater induced by the electrodes. Indeed, electrolysis of the anode might release metal-ions in the environment and a secondary production of oxidants such as chlorine and bromine may occur (Stewart, 1972; Yalçin et al., 1997). Oxidants including ozone are known to delay or reduce the hatchability of cod eggs (Grotmol et al., 2003). They may modify the protein polymer compounds in the eggshell rendering it more resistant to hatching enzymes responsible for the weakening of this membrane. The secretion of these enzymes by the hatching gland may also be inhibited. In addition, low concentrations of possibly produced metal-ions and oxidants are well known to be toxic and reduce the survival of aquatic organisms (Stewart et al., 1979; Abarnou and Miossec, 1992; Arimoto et al., 1996). However, in the present study, electrolysis was probably minor because exposures of only 5 seconds were used and no differences in survival rate were observed at 2 DPH. Additionally, at sea this phenomenon will be limited by the continuous abrasion of the electrode surface during towing at speeds of 3 knots (Stewart, 1972). Nevertheless, different chemical reactions might still be possible in the electrically trawled sediment, especially in substrates rich in organic matter and metals (Alvarez-Iglesias and Rubio, 2009; Soetaert et al. 2015). Another explanation for the delayed hatching rate could be that electrical pulses might interfere with the frequency of sporadic muscular contractions that finally cause the chorion to tear (Hall et al., 2004).

The vulnerability of early life stages seems to decrease as their development proceeds. However, for some fresh water fish species, the above mentioned sensitive embryonic stages appear to be less susceptible to electrical stimulation than later post-hatching stages (Henry et al. 2003, Henry and Grizzle, 2003, 2006; Muth and Ruppert, 1997). In our experiment, a significantly lower survival rate was noted following exposure of larvae at 2 and 26 DPH in comparison with their corresponding control group. In this latter stage, many organ systems are developing (Yin and Blaxter 1986; Pedersen and Falk-Petersen, 1992; Brown et al., 2003). Additionally, this stage is more sensitive to external stress because of its transition from cutaneous to gill respiration (Herbing et al., 1996). Besides, larvae are feeding on *Artemia* and need to chase their prey actively with their yolk completely depleted. In general, this developmental stage is known to be a bottleneck in cod larviculture conditions with failure to initiate and maintain sufficient feeding being the major source leading to mass mortality (Puvanendran and Brown, 1999, 2002; Brown et al., 2003). In contrast, cod at later developmental stages, larvae in metamorphosis and juveniles display higher survival rates and appear to be more robust (Pedersen and Falk-Petersen, 1992; Opstad et al., 2006; Meier et al., 2010). Indeed, in our studies, no differences in survival were found during metamorphosis or in the juvenile stage of cod exposed at 46 or 60 DPH, respectively.

In the present study, a homogeneous electric field of approximately 150V/m for 5 s was applied as a worst-case scenario to randomly orientated animals. Orientation and position in the electrical field are important as the highest head to tail voltage will be experienced when animals are orientated perpendicular to the electrodes. At sea, a heterogeneous electrical field distribution is created for less than 2 seconds on the assumption that individuals are at rest and only exposed when 150 cm long electrodes are passing by at a speed of 3 knots. A heterogeneous field implies that field strengths are higher in close proximity (up to 150 V/m at 5 cm) and lower when the distance to the electrode increases (approximately 30 V/m at a moderate distance of 30 cm) (Verschueren and Polet 2009; Verschueren et al., 2013). The latter is presumed to be the case for

the majority of organisms. Indeed, the potential for cod embryos and larvae to be exposed to an electrical field of 150 V/m during electrofishing will be low as they are pelagic and buoyant (Fahay, 1983; Markle and Frost, 1985) while the electrical field will be limited to the net opening of the trawl. However, turbulent forces such as mixing forces from wind may distribute pelagic life stages in a downward direction increasing their chances for contact with the electrical field (Sundby, 1983; Conway et al., 1997). Therefore, young buoyant life stages of cod may have higher chances of contact with the electrical field in their shallow coastal spawning areas (Munk et al., 2002) where shrimp trawling often occurs. Cod larvae move deeper as they become older (Yin and Blaxter, 1987; Heesen and Rijnsdorp, 1989) and descend from the water column to bottom habitats at sizes of 2.5-6 cm, when a complete transformation to the juvenile stage occurs (Fahay 1983; Lough et al., 1989). Thus, it is more likely that these developmental stages will be in contact with the electrofishing equipment. However, no significant differences in mortality compared to controls were noted for cod exposed during or after metamorphosis to the young juvenile stage at 46 and 60 DPH, respectively. Nevertheless, the impact on older juveniles larger than 2.4 cm was not examined in our trials.

No significant differences in morphometric parameters between exposed and control organisms were found, indicating that growth rate was not affected by electric field exposure. Furthermore, morphometric changes, such as jaw deformities which are known to prevent feeding (Tilseth et al., 1984; Meier et al., 2010), abnormal yolk resorption, increased incurvation or deformations, such as lordosis and scoliosis, did not differ between exposed and control groups.

Eggs were obtained from three batches to ensure that all embryos, larvae and juveniles to be compared were the same age in each experiment to reduce variability in hatching percentage and egg and larval quality between replicates. Indeed, inconsistency in growth rates and survival among tanks is one of the major problems encountered with intensive cod larval rearing (Thorsen et al., 2003; Hamre, 2006; Monk et al., 2006). These phenomena introduce complications in interpreting results from studies of fish larvae as was the case in the present study for the larvae

exposed at 2 and 11 DPH. We are hesitant to draw any conclusions from these data especially since the differences in survival were borderline significant. This is in contrast with the findings for the 26 DPH larvae, where the difference between exposed and control groups was much greater.

The present research is innovative in that it is the first to examine the impact of electrical pulses on a marine fish species during its embryonic, larval and young juvenile stages employing cod as a model species. However, follow-up studies are necessary to fully grasp the potential impact of pulse trawls on these young life stages and reproductive success of adults (Cho et al., 2002). Indeed, studies on the impact of electrical pulses on the reproduction of adult brood stock and on fertility success of exposed gametes are lacking. Exposure of ripe female fish to electrical fields may cause significant damage or premature expulsion of gametes and reduced viability of subsequently fertilized eggs (Muth and Ruppert 1997; Roach 1999). Therefore, a greater proportion of abnormal cod larvae hatching from eggs of stressed females may be produced (Morgan et al., 1999). Although multiple exposures with intervals of 1-5 min did not appear to cause major harm to zebrafish embryos (Nutile et al., 2012), research on the effects of electrofishing on young marine organisms is limited to single exposure events. Information on the impact of multiple exposures is important, as certain fishing grounds including spawning areas may be fished intensely during particular seasonal periods (Piet and Hintzen, 2012; van Denderen, 2015). In addition, other marine species should be included such as flatfish. During larval development, these fish species demonstrate very complex morphological changes during metamorphosis, such as migration of the eye (Palazzi et al., 2006; Piccinetti et al., 2012) and could therefore be more vulnerable to electric pulses. Other species, such as herring, also need to be investigated since demersal eggs are produced (Yin and Blaxter, 1987) which could be exposed when electrodes are towed over the sea bed.

Acknowledgments:

This research received funding and support from the Agency for Innovation by Science and Technology in Flanders (IWT), The European Fisheries fund (EFF), Aquaexcel and the staff at the Centre for Marine Aquaculture Research in Tromsø.

REFERENCES

- Abarnou, A., and L. Miossec. 1992. Chlorinated waters discharged to the marine environment chemistry and environmental impact. An overview. The Science of the Total Environment 126: 173-197.
- Alvarez-Iglesias, P., and B. Rubio. 2009. Redox status and heavy metal risk in intertidal sediments in NW Spain as inferred from the degrees of pyritization of iron and trace elements. Marine Pollution Bulletin 58: 542–551.
- Andersen, O. G. N., J. R. Nielsen, and E. L. B. Smidt. 1994. Description and comparison of eggs and yolk sac larvae of Greenland cod (*Gadus ogac*), Atlantic cod (*Gadus morhua*) and Arctic cod (*Boreogadus saida*) from West Greenland waters. Journal of Northwest Atlantic Fishery Science 16: 19-32.
- Auditore, P. J., R. G. Lough, and E. A. Broughton. 1994. A review of the comparative development of Atlantic cod (*Gadus morhua* L.) and Haddock (*Melanogrammus aeglefinus* L.) based on an illustrated series of larvae and juveniles from the Georges Bank. Northwest Atlantic Fisheries Organization Scientific Council Studies 20: 7-18.
- Arimoto, M., J. Sato, K. Maruyama, G. Mimura, and I. Furusawa. 1996. Effect of chemical and physical treatments on the inactivation of striped jak nervous necrosis virus (SJNNV). Aquaculture 143:15-22.
- Bohl, R. J., T. B. Henry, and R. J. Strange. 2010. Electroshock-induced mortality in freshwater fish embryos increases with embryo diameter: a model based on results from 10 species. Journal of Fish Biology 76: 975–986.
- Boonstra, G., and S. De Groot. 1974. The development of an electrified shrimp-trawl in the Netherlands. ICES Journal of Marine Science/ Journal du Conseil 35: 165-170.

- Brown, J. A., G. Minkoff, V., Puvanendran. 2003. Larviculture of Atlantic cod (*Gadus morhua*): Progress, protocols and problems. Aquaculture 227: 357-372.
- Catchpole, T. L., A. S. Revill, J. Innes, and S. Pascoe. 2008. Evaluating the efficacy of technical measures: a case study of selection device legislation in the UK *Crangon crangon* (brown shrimp) fishery. ICES Journal of Marine Science/ Journal du Conseil 65: 267-275.
- Chen, C., S.W. Smye, M. P. Robinson, and J. A. Evans. 2006. Membrane electroporation theories: a review. Medical and Biological Engineering and Computing 44: 5–14.
- Cho, G. K., J. W. Heath, and D. D. Heath. 2002. Electroshocking influences chinook salmon egg survival and juvenile physiology and immunology. Transactions of the American Fisheries Society 131: 224–233.
- Conway, D. V. P., S. H. Coombs, and C. Smith. 1997. Vertical distribution of fish eggs and larvae in the Irish Sea and southern North Sea. ICES Journal of Marine Science 54: 136–147.
- Desender, M., K. Chiers, H. Polet, B. Verschueren, J. H. Saunders, B. Ampe, A. Mortensen, V. Puvanendran, and A. Decostere. 2016. Short-term effect of pulsed direct current on various species of adult fish and its implication in pulse trawling for brown shrimp in the North Sea. Fisheries Research 179: 90-97.
- Desender, M., S. Kajiura, B. Ampe, L. Dumolein, H. Polet, K. Chiers, and A. Decostere. 2017. Pulse trawling: Evaluating its impact on prey detection by small-spotted catshark (*Scyliorhinus canicula*). Journal of Experimental Marine Biology and Ecology 486: 336–343
- Dwyer, W. P., W. A. Fredenburg, and D. A. Erdahl. 1993. Influence of electroshock and mechanical shock on survival of trout eggs. North American Journal of Fisheries Management 13: 839–843.
- Dwyer, W. P., and D. A. Erdahl. 1995. Effects of electroshock voltage, wave form, and pulse rate on survival of cutthroat trout eggs. North American Journal of Fisheries Management 15: 647–650.
- EU, 1998. Council Regulation (EC) No. 850/98 of 30 march 1998 for the conservation of fishery resources through technical measures for the protection of juveniles of marine organisms (OJL 125, 27.4.1998), 55pp.
- EU, 2009. Council Regulation (EC) No. 43/2009 of 16 January 2009 fixing for 2009 the fishing opportunities and associated conditions for certain fish stocks and groups of fish stocks, applicable in Community waters and, for Community vessels, in water where catch limitations are required. (OJ L22, 26.1.2009), 205pp.

- EU, 2013. Art 14 of the Eu Regulation No 1380/2013 of the European Parliament and of the Council of 11 December 2013 on the Common Fisheries Policy, amending Council Regulations (EC) No 1954/2003 and (EC) No 1224/2009 and repealing Council Regulations (EC) No 2371/2002 and (EC) No 639/2004 and Council Decision 2004/585/EC.
- Fahay, M.P. 1983. Guide to the early stages of marine fishes occurring in the western North Atlantic Ocean, Cape Hatteras to the southern Scotian Shelf. Journal of Northwest Atlantic Fishery Science 4: 1-423.
- FAO, 2009. Fisheries management. The ecosystem approach to fisheries. FAO Technical Guidelines for Responsible Fisheries, 4 (Suppl. 2). 112 pp. ISBN 978-92-5-106424-5
- FAO, 2011. Code of conduct for responsible fisheries. FAO publication 108pp. ISBN 978-92-5-106708-6
- FAO, 2012. State of world fisheries and aquaculture. 230pp. ISBN 978-92-5-107225-7
- Gaylor, D. C., K. Prakahasante, and R. C. Lee. 1988. Significance of cell-size and tissue structure in electrical trauma. Journal of Theoretical Biology 133: 223–237.
- Glauert, A. M. 1987. Fixation, Dehydration and Embedding of Biological Specimens, 1st edn. Amsterdam: Elsevier Science.
- Godfrey, H. 1957. Mortalities among developing trout and salmon ova following shock by direct-current electric fishing gear. Journal of the Fisheries Research Board of Canada 14: 153–164.
- Grotmol, S., E. Dahl-Paulsen, and G. K. Totland. 2003. Hatchability of eggs from Atlantic cod, turbot and Atlantic halibut after disinfection with ozonated seawater. Aquaculture 221: 245–254.
- 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.
- Haan, D., J. Fosseidengen, P. Fjelldal, and D. Burggraaf. 2011. The effect of electric pulse stimulation to juvenile cod and cod of commercial landing size, IMARES Wageningen UR C141/11: 44p.
- Haan, D., B. Marlen, T. Kristiansen, and J. Fosseidengen. 2009. The effect of pulse stimulation on biota: research in relation to ICES advice: progress report on the effects on cod, IMARES Wageningen UR C098/08: 25p.
- Hall, T. E., P. Smith, and I. A. Johnston. 2004. Stages of embryonic development in the Atlantic cod *Gadus Morhua*. Journal of Morphology 259: 255-270.

- Hamre, K. 2006. Nutrition in cod (*Gadus morhua*) larvae and juveniles. Ices Journal of Marine Science 63: 267–274.
- Hansen, Ø. J., and V. Puvanendran. 2010. Fertilization success and blastomere morphology as predictors of egg and juvenile quality for domesticated Atlantic cod, *Gadus morhua*, broodstock. Aquaculture Research 41:1791-1798.
- Hansen, Ø. J., V. Puvanendran, and R. Bangera. 2015. Do maternal age and experience contribute to better growth, survival and disease resistance of offspring in Atlantic cod (*Gadus morhua*)? Aquaculture International 23: 1157–1164.
- Hayes, F. R. 1949. The growth, general chemistry, and temperature relations of salmonid eggs. The Quarterly Review of Biology 24: 281–308.
- Heesen, H. J. L., and A. D. Rijnsdorp. 1989. Investigation on egg production and mortality of cod (*Gadus morhua* L.) and plaice (*Pleuronectes platessa* L.) in the southern and eastern North Sea in 1987 and 1988. Rapp. P. v. Réun. Cons. int. Explor. Mer 191: 15-20.
- Henry, T. B., and J. M. Grizzle. 2003. Electroshocking-induced injuries in newly transformed juvenile fish. Journal of Aquatic Animal Health 15: 147–157.
- Henry, T. B., J. M. Grizzle, and M. Maceina. 2003. Electrofishing-induced mortality of four fish species during posthatching development. Transactions of the American Fisheries Society 132: 299–306.
- Henry, T. B., and J. M. Grizzle. 2004. Survival of largemouth bass, bluegill and channel catfish embryos after electroshocking. Journal of Fish Biology 64: 1206–1216.
- Henry, T. B., and J. M. Grizzle. 2006. Electric-induced mortality of newly transformed juvenile fishes in waters of different conductivity. Journal of Fish Biology 68: 747–758.
- Herbing, I. H. V., T. Miyake, B. K. Hall, and R. G. Boutilier. 1996. Ontogeny of feeding and respiration in larval Atlantic cod *Gadus morhua* (Teleostei, Gadiformes): I. Morphology. Journal of Morphology 227: 15-35.
- Jennings, S., and M. J. Kaiser. 1998. The effects of fishing on marine ecosystems. Advances in Marine Biology 34: 221–233.
- Keefe, M., T. A. Whitesel, and P. Angelone. 2000. Induced mortality and sub-lethal injuries in embryonic brook trout from pulsed DC electroshocking. North American Journal of Fisheries Management 20: 320–327.

- Kolz, A. L., and J. B. Reynolds. 1990. Principles and techniques of electrofishing. Outline manual for course, Leetown, US, Fish and Wildlife Service Fisheries Academy.
- Lamarque, P. 1990. Electrophysiology of fish in electric fields. In: Fishing with Electricity, Applications in Freshwater Fisheries (eds I.G.L. Cowx and P. Lamarque). Fishing News Books, Blackwell Scientific Publications, Oxford pp. 4–33.
- Lindeboom, H. J., and S. J. de Groot. 1998. The effects of different types of fisheries on the North Sea and Irish Sea benthic eco-systems. EU-project AIR2-CT94-1664(IMPACT-II), Final Report ISSN 0923-3210, 404 pp.
- Lough, R. G., P. C. Valentine, D. C. Potter, P. J. Auditore, G. R. Bolz, J. D. Neilson, and R. I. Perry. 1989. Ecology and distribution of juvenile cod and haddock in relation to sediment type and bottom currents on eastern Georges Bank. Marine Ecology Progress Series 56: 1-12.
- Markle, D. F., and L. A. Frost. 1985. Comparative morphology, seasonality, and a key to the planktonic fish eggs from the Nova Scotian shelf. Canadian Journal of Zoology 63: 246-257.
- Meier, S., H. C. Morton, G. Nyhammer, B. E. Grøsvik, V. Makhotin, A. Geffen, S. Boitsov, K. A. Kvestad, A. Bohne-Kjersem, A. Goksøyr, A. Folkvord, J. Klungsøyr, and A. Svardal. 2010. Development of Atlantic cod (*Gadus morhua*) exposed to produced water during early life stages: Effects on embryos, larvae, and juvenile fish. Marine Environmental Research 70: 383-394.
- Monk, J., V. Puvanendran, and J. A. Brown. 2006. Do different light regimes affect the foraging behaviour, growth and survival of larval cod (*Gadus morhua* L.)? Aquaculture 257: 287-293.
- Morgan, M.J., C. E. Wilson, and L. W. Crim. 1999. The effect of stress on reproduction in Atlantic cod. Journal of Fish Biology 54: 477-488.
- Munk, P., P. J. Wright, and N. J. Pihl. 2002. Distribution of the early larval stages of cod, plaice and lesser sandeel across haline fronts in the North Sea. Estuarine, Coastal and Shelf Science 55: 139–149.
- Muth, R. T., and J. B. Rupert. 1997. Effects of electrofishing fields on captive embryos and larvae of razorback sucker. North American Journal of Fisheries Management 17: 160–166.
- Nikolakakis, S., P. Bossier, G. Kanlis, K. Dierckens, and D. Adriaens. 2014. Protocol for quantitative shape analysis of deformities in early larval European seabass *Dicentrarchus labrax*. Journal of Fish Biology 84: 206–224.
- Nutile, S., J. J. Amberg, M. S. Sepulveda, and R. R. Goforth. 2012. Effects of multiple electrical field exposures on Cyprinid embryo survival. North American journal of fisheries management 32: 875-879.

- Opstad, I., J. Suontama, E. Langmyhr, and R. E. Olsen. 2006. Growth, survival, and development of Atlantic cod (*Gadus morhua* L.) weaned onto diets containing various sources of marine protein. ICES Journal of Marine Science 63: 320-325.
- Palazzi, R., J. Richard, G. Bozzato, and L. Zanella. 2006. Larval and juvenile rearing of common sole (*Solea solea* L.) in the Northern Adriatic (Italy). Aquaculture 255: 495-506.
- Paschen, M., U. Richter, and W. Köpnick. 2000. TRAPESE—Trawl Penetration in the Seabed. Final Report EU Contract 96-006, University of Rostock.
- Pedersen, T., and I. B. Falk-Petersen. 1992. Morphological changes during metamorphosis in cod (*Gadus morhua* L.), with particular reference to the development of the stomach and pyloric caeca. Journal of Fish Biology 41: 449-461.
- Piccinetti, C. C., L. A. Ricci, N. Tokle, G. Radaelli, F. Pascoli, L. Cossignani, F. Palermo, G. Mosconi, V. Nozzi, F. Raccanello, and I. Olivotto. 2012. Malnutrition may affect common sole (*Solea solea* L.) growth, pigmentation and stress response: Molecular, biochemical and histological implications. Comparative biochemistry and physiology Part A 161: 361-371.
- Piet, G. J., and N. T. Hintzen. 2012. Indicators of fishing pressure and seafloor integrity. ICES Journal of Marine Science 69: 1850–1858.
- Polet, H. 2002. Selectivity experiments with sorting grids in the North Sea brown shrimp (*Crangon crangon*) fishery. Fisheries Research 54: 217-233.
- Polet, H., F. Delanghe, and R. Verschoore. 2005a. On electrical fishing for brown shrimp (*Crangon crangon*): I. Laboratory experiments. Fisheries Research 72: 1-12.
- Polet, H., F. Delanghe, and R. Verschoore. 2005b. On electrical fishing for brown shrimp (*Crangon crangon*): II. Sea trials. Fisheries Research 72: 13-27.
- Puvanendran, V., and J. A. Brown. 1999. Foraging, growth and survival of Atlantic cod larvae reared in different prey concentrations. Aquaculture 175: 77–92.
- Puvanendran, V., and J. A. Brown. 2002. Foraging, growth and survival of Atlantic cod larvae reared in different light intensities and photoperiods. Aquaculture 214: 131-151.
- Rasenberg, M., H. van Overzee, F. Quirijns, M. Warmerdam, B. van Os, and C. Rink. 2013. Monitoring catches in the pulse fishery. Imares Wageningen UR, Report number C122/13.
- Revill, A. S., and R. Holst. 2004. Reducing discards of North Sea brown shrimp (*C. crangon*) by trawl modification. Fisheries Research 68: 113-122.

- Roach, S. M. 1999. Influence of electrofishing on the mortality of Arctic grayling eggs. North American Journal of Fisheries Management 19: 923–929.
- Rollefsen, G. 1930. The Susceptibility of Cod Eggs to external Influences. Norwegian Bureau of Fisheries, Bergen.
- Scientific, Technical and Economic Committee for Fisheries (STECF). 2014. Evaluation of Fishing Effort Regimes in European Waters - Part 2. Publications Office of the European Union, Luxembourg, EUR 27027 EN, JRC 93183, 8 44 pp.
- Snyder, D.E. 2003. Electrofishing and its harmful effects on fish. U.S. Geological Survey, Information and Technology Report 2003-0002.
- 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: Journal du Conseil 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.
- Stewart, P. A. M. 1972. The selection of electrode materials for electrical fishing. ICES Document IR 72–1. 12 pp.
- Stewart, M. E., W. J. Blogoslawski, R. Y. Hsu, and G. R. Helz. 1979. By-products of oxidative biocides: Toxicity to oyster larvae. Marine pollution bulletin 10: 166-169.
- Stroup, W. 2012. Generalized Linear Mixed Models: Modern Concepts, Methods and Applications. CRC Press.
- Sundby, S. 1983. A one-dimensional model for the vertical distribution of pelagic fish eggs in the mixed layer. Deep-Sea Research 30: 645-661.
- Thorsen, A., E. Trippel, and Y. Lambert. 2003. Experimental methods to monitor the production and quality of eggs of captive marine fish. Journal of Northwest Atlantic Fisheries science 33: 55-70.
- Tilseth, S., T. S. Solberg, and K. Westrheim. 1984. Sublethal effects of the water-soluble fraction of Ekofisk crude oil on early larval stages of cod (*Gadus morhua* L.). Marine Environmental Research 11: 1-16.
- van Denderen, P. D., N. T. Hintzen, T. van Kooten, and A. D. Rijnsdorp. 2015. Temporal aggregation of bottom trawling and its implication for the impact on the benthic ecosystem. – ICES Journal of Marine Science 72: 952–961.

- van Marlen, B., J. 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.
- Verschueren, B., and H. Polet. 2009. Research summary on HOVERCRAN, hovering pulse trawl for a selective *Crangon* fishery. Institute for Agricultural and Fisheries Research, Report nr.: 23.
- Verschueren, B., B. Vanelslander, and H. Polet. 2012. Verduurzaming van de Garnalenvisserij met de Garnalenpuls. Institute for Agricultural and Fisheries Research, Report nr.: 116. 102pp.
- Verschueren, B., and B. Vanelslander. 2013. Project Combipuls: Pulsvisserij op tong en garnaal. Institute for Agricultural and Fisheries Research. 74pp.
- Verschueren B., H. Lenoir, L. Vandamme, and B. Vanelslander. 2014. Evaluatie van een seizoen pulsvisserij op garnaal met HA 31. Institute for Agricultural and Fisheries Research, Report nr.: 157. 104pp.
- Yalçin, H., T. Koç, V. Pamuk. 1997. Hydrogen and bromine production from concentrated sea-water. Int. Journal of Hydrogen Energy 22: 967-970
- Yamagami, K. 1988. Mechanisms of hatching in fish. Fish Physiology 11: 447–499.
- Yin, M. C., and J. H. S. Blaxter. 1987. Temperature, salinity tolerance, and buoyancy during early development and starvation of Clyde and North Sea herring, cod, and flounder larvae. J. Exp. Mar. Biol. Ecol. 107: 279-290.
- Yu, C., Z. Chen, L. Chen, and P. He. 2007. The rise and fall of electrical beam trawling for shrimp in the East China Sea: technology, fishery, and conservation implications. ICES Journal of Marine Science: Journal du Conseil 64: 1592-1597.

FIGURE & TABLE CAPTIONS

Figure 1: Two plate shaped electrodes connected to the pulse generator created a homogeneous electric field to which specimens were exposed.

Figure 2: Overview of the measurements taken on 1 DPH larva; 1) Straight notochord length; 2) Eye diameter; 3) Head height; 4) Muscle height; 5) Total notochord length and 6) Yolk surface

Figure 3: Survivorship of cod embryos exposed on 1, 5 and 18 DPF.

Figure 4: Hatching rate of cod embryos at 21 DPF (0 DPH) that were exposed to electric current at 1, 5 and 18 DPF.

Figure 5: Short-term survival rates of cod larvae exposed at 2, 11, 26, 46 DPH and as juveniles (60 DPH) to an electric field and unexposed controls at the same time points.

Table 1: Overview of the different experiments across three batches and eight developmental stages of Atlantic cod showing when samples were taken for exposure to electric current, survival and morphometric analysis. DPF (days post fertilisation), DPH (days post hatching). *50% of embryos hatched at 21 DPF = 0 DPH.

Experiment	Batch	Developmental stage	Exposure	Survival	1st morphometric sample point	2nd morphometric sample point
1	1	Embryo*	1 DPF	2 DPH	2 DPH	22 DPH
2	1	Embryo*	5 DPF	2 DPH	2 DPH	22 DPH
3	1	Embryo*	18 DPF	2 DPH	2 DPH	22 DPH
4	2	Larvae	2 DPH	9 DPH	3 DPH	26 DPH
5	2	Larvae	11 DPH	18 DPH	12 DPH	27 DPH
6	2	Larvae	26 DPH	33 DPH	27 DPH	58 DPH
7	2	Larvae	46 DPH	53 DPH	47 DPH	64 DPH
8	3	young juvenile	60 DPH	89 DPH	61 DPH	89 DPH

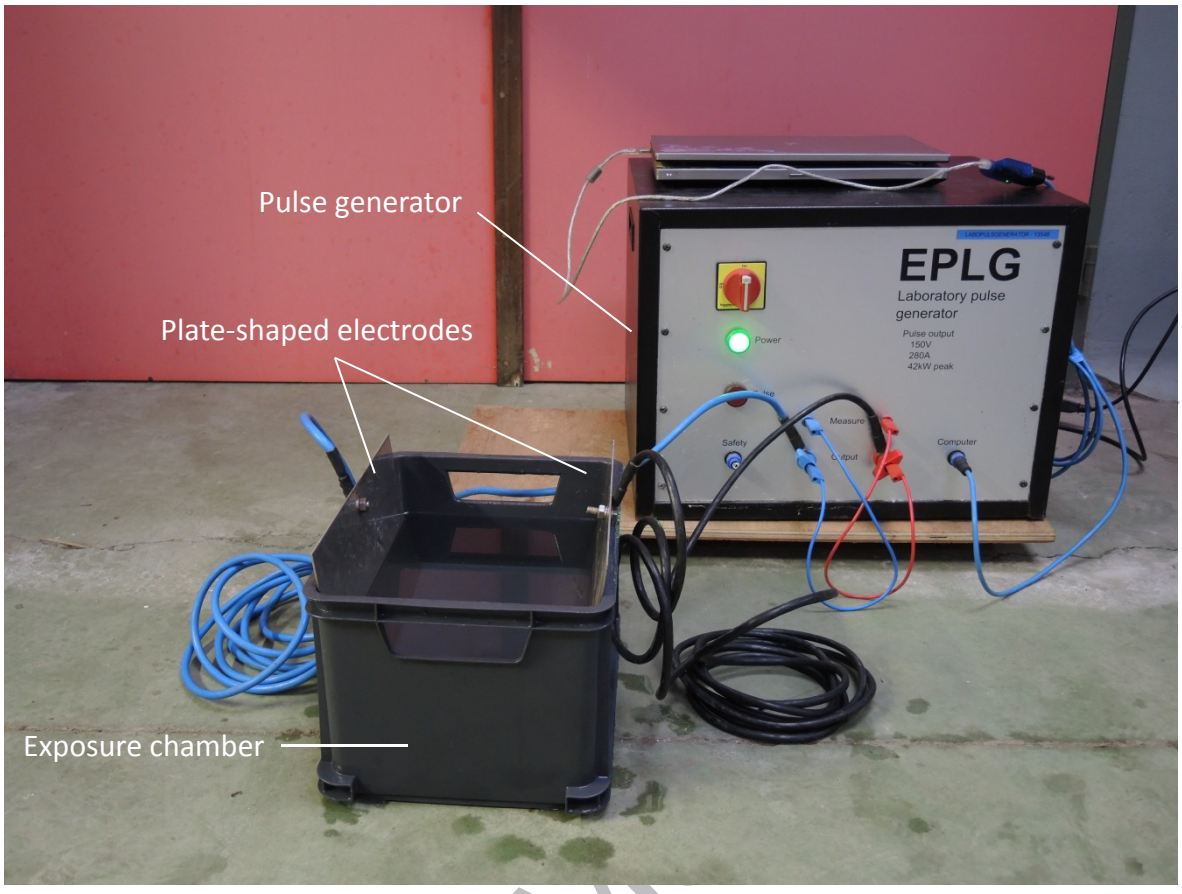

Figure 1

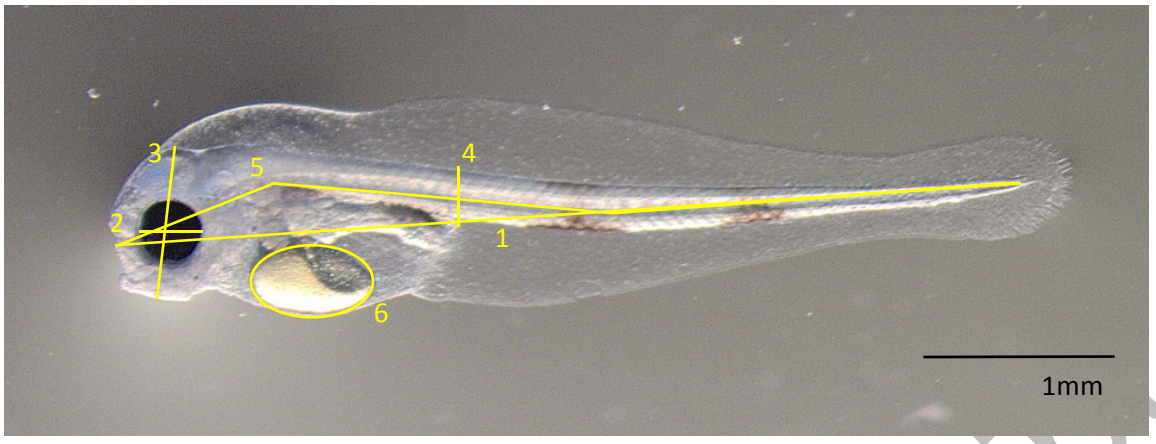

Figure 2

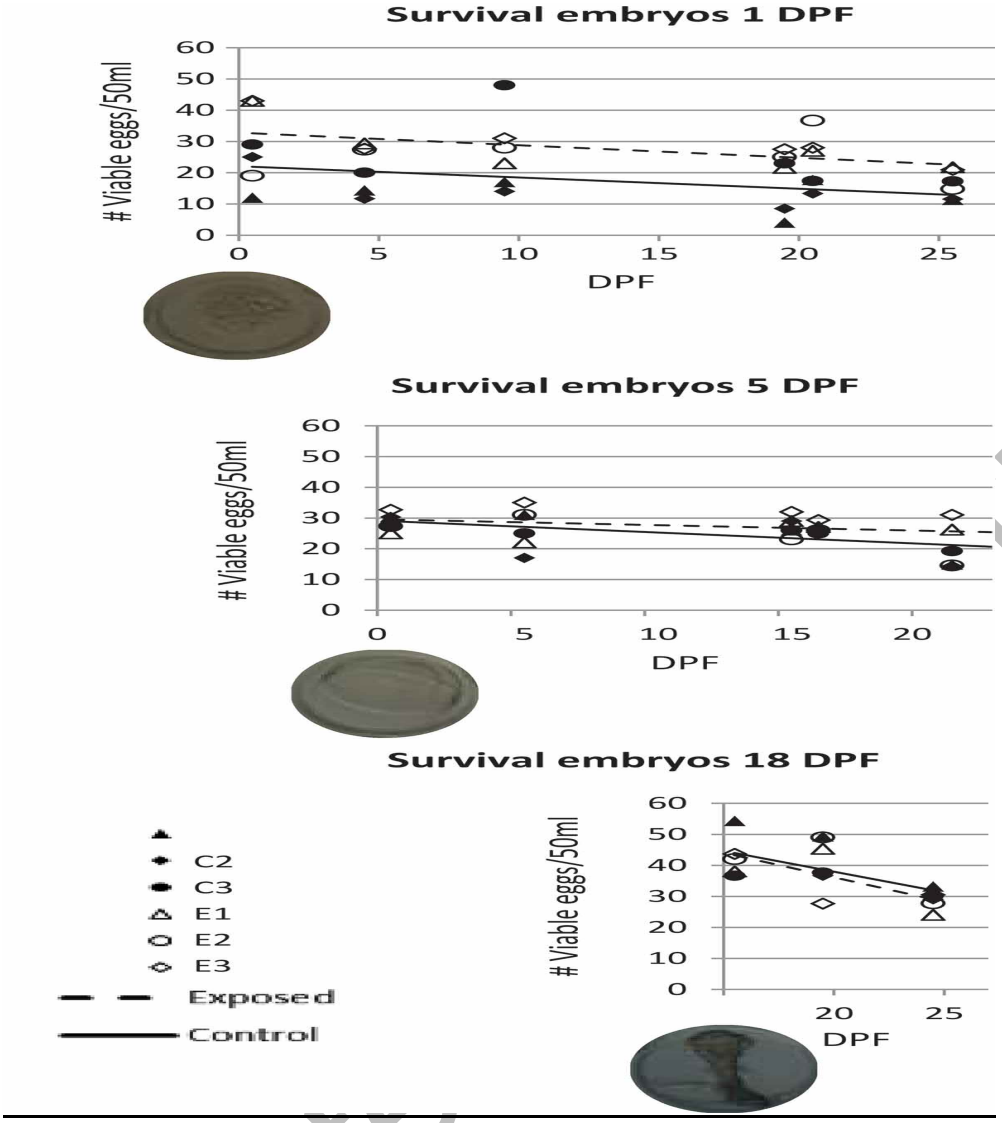

Figure 3

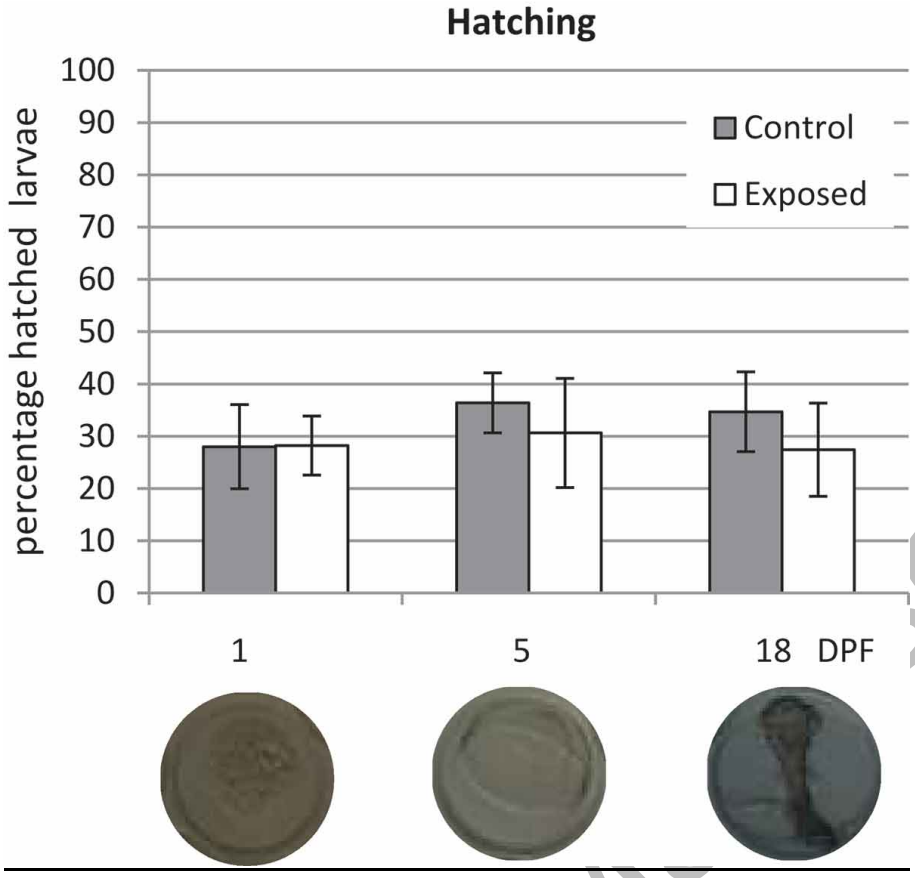

Figure 4

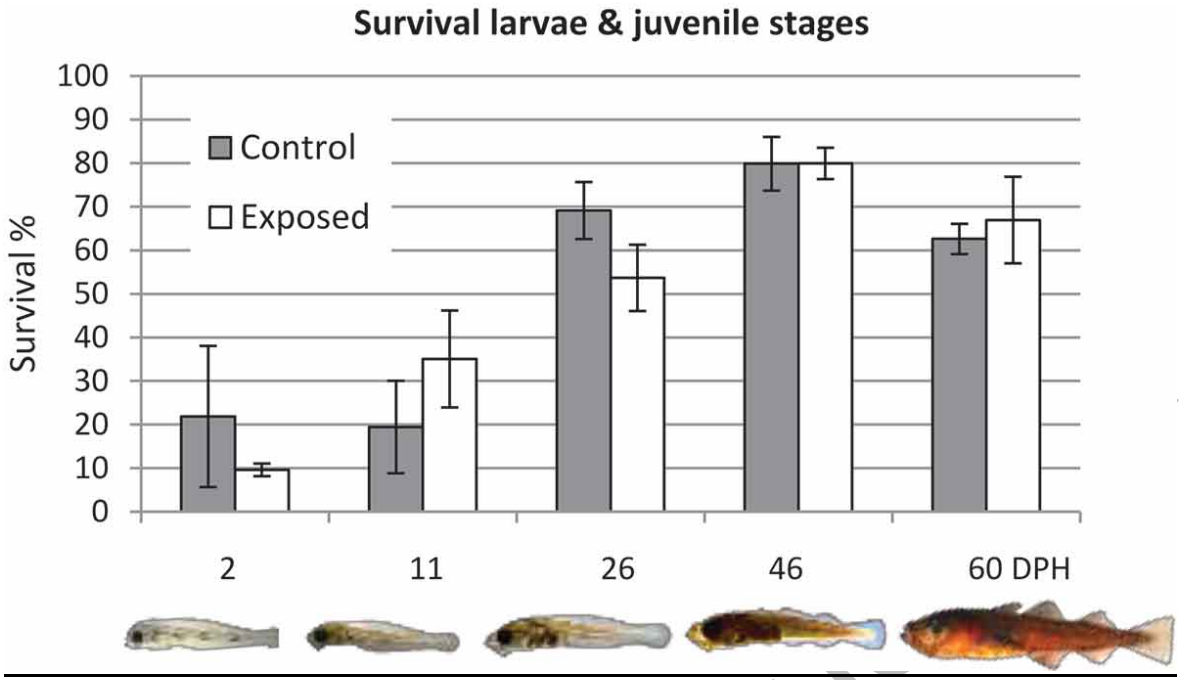

Figure 5