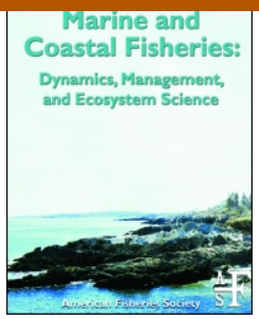

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ARTICLE

Impact of Pulsed Direct Current on Embryos, Larvae, and Young Juveniles of Atlantic Cod and its Implications for Electrotrawling of Brown Shrimp

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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 *Crangon crangon*, an electrical field selectively induces a startle response in the shrimp. Other benthic organisms remain mainly on the seafloor and escape underneath a hovering trawl. Previous experiments have indicated that this pulse has no short-term major harmful effects on adult fish and invertebrates. However, the impact on young marine life stages is still unknown. Because brown shrimp are caught in shallow coastal zones and estuaries, which serve as important nurseries or spawning areas for a wide range of marine species, electrotrawling on these grounds could harm embryos, larvae, and juveniles. We carried out experiments with different developmental stages of Atlantic Cod *Gadus morhua*, which are considered vulnerable to electrical pulses. Three embryonic stages, four larval stages, and one juvenile stage of Atlantic Cod were exposed to a homogeneous electrical field of 150 V_{peak}/m for 5 s, mimicking a worst-case scenario. We detected no significant differences in embryo mortality rate between control and exposed groups. However, for the embryonic stage exposed at 18 d postfertilization, the initial hatching rate was lower. Larvae that were exposed at 2 and 26 d posthatch exhibited higher mortality rates than the corresponding nonexposed control 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, or head. Although exposure to a worst-case electrical field did not impact survival or development for six of the eight young life stages of Atlantic Cod, the observed delayed hatching rate and decreased survival for larvae might indicate an impact of electric pulses and warrant further research.

Beam trawls are used extensively in the North Sea to catch brown shrimp *Crangon crangon* and flatfishes, particularly Sole *Solea solea* and Plaice *Pleuronectes platessa* (STECF 2014). However, this demersal fishing technique negatively impacts the marine environment, with high bycatch rates, intense bottom contact, and high fuel consumption as major drawbacks (Jennings and Kaiser 1998; Lindeboom and de Groot 1998; Paschen et al. 2000). To deal with the upcoming discard ban and contribute to a more ecosystem-based approach in fisheries management (FAO 2009, 2012), measures that mitigate the disadvantages of traditional beam trawling by reducing seabed contact and enhancing selective fishing are sought (Polet 2002; Revill and Holst 2004; Catchpole et al. 2008). Electrical pulse fishing offers a promising alternative technique for meeting these requirements (Boonstra and de Groot 1974; Polet et al. 2005a; Soetaert et al. 2015). Using such 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 gear for targeting brown shrimp uses a 5-Hz, low-frequency pulsed direct current (PDC) of 0.5 ms, creating a field strength of at least 30 V/m between two thread-shaped electrodes placed in parallel at a distance of 60 cm. In this way, a startle response (i.e., 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). Thus, all 36 bobbins that are attached to the groundrope of a conventional trawl and used to mechanically startle the shrimp and protect the ground gear are removed from the original electrotrawl for brown shrimp (the Hovercran; Verschueren and Polet 2009; Verschueren et al. 2012). However, at sea, vessels differ in rigging, gear configuration, and the 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 during 2013. However, 11 bobbins were also implemented on a modified straight bobbin rope instead of the 36 bobbins and the U-shaped bobbin rope used in traditional gear. A 76% decrease in discard amount and a 60% reduction in seabed contact (resulting in 23% less drag resistance) were noted (Verschueren et al. 2014). Furthermore, the catch volume of commercial shrimp in summer was increased by 16%, especially in clear water with low turbidity and during daylight. For these reasons, the use of electrical pulses in fishing gear is regarded as a promising fishing method from an economic and environmental point of view.

Fishing with electricity in the sea has been prohibited since 1998 in Europe (European Council 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 (European Council 2009). In 2013, 42 additional licenses were allocated to Dutch fisheries (European Council 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). Introduction of a fishing method based on this technology without a sound knowledge of the interactions between pulse fishing and both target and nontarget marine organisms would violate the principles of responsible fishing (FAO 2011).

Previously, in short-term studies under 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; Soetaert et al. 2014; Desender et al. 2016, 2017). Still, the potential impact on young life stages is a growing concern that has not been addressed.

Because electrofishing is a commonly used sampling technique in rivers, ponds, and lakes, research on the impacts of electrical currents on eggs, larvae, and juveniles has previously focused on freshwater species. Indeed, electrical fields could negatively affect young organisms, with the intensity and type of electrical field, exposure duration, developmental stage, and species as determining parameters (Snyder 2003). However, data resulting from the use of electrical currents applied in freshwater with different electrical settings cannot necessarily be extrapolated to the marine environment due to differences in conductivity. The field intensity used is 2–6 times higher and the duration is 10–60 times longer in freshwater than in seawater. Given that intensity and duration are the most critical parameters affecting embryos and larvae (Dwyer et al. 1993; Dwyer and Erdahl 1995), it might be assumed that the effects of electrical currents would be more moderate in seawater than in freshwater (Soetaert et al. 2015). Whether the latter hypothesis is correct needs to be empirically investigated. Indeed, studies addressing the effects of electrofishing on young life stages of marine species are not available. Nevertheless, such data are crucial, as brown shrimp are often caught in shallow coastal zones adjacent to extensive tidal flat areas (e.g., the Wadden Sea), which are often important nurseries and spawning areas for a wide range of marine species.

The present study is the first to expose Atlantic Cod *Gadus morhua* at various embryonic, larval, and young juvenile stages to electrical pulses like those used in targeting brown shrimp and to evaluate their survival. Exposure might not cause a significant increase in mortality of these early life stages 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. The Atlantic Cod was adopted as a model organism for marine coldwater roundfish species. This commercially important top predator is considered vulnerable to high-frequency electrical pulses based on observations of commercial catches onboard flatfish pulse trawlers (Rasenberg et al. 2013; van Marlen et al. 2014) and based on the results of laboratory experiments (Haan et al. 2009, 2011, 2016).

METHODS

Experimental Animals

Fertilized eggs from strip-spawned captive broodstock in three different spawning events (batches) maintained at the Centre for Marine Aquaculture Research (Norwegian Institute of Food, Fisheries, and Aquaculture Research [NOFIMA], Tromsø, Norway) were incubated until the desired developmental stage. When approximately 50% of hatching had occurred, this was designated as 0 d posthatch (DPH) in larval age. In total, eight developmental stages were exposed, resulting in eight experiments conducted from early cleavage in the embryonic stages (batch 1) to larval stages (batch 2) to postmetamorphic juveniles (batch 3). An overview of the experiments is illustrated in Table 1 and Supplementary Figure S.1 (available separately online). Three stages of embryos at 1, 5, and 18 d postfertilization (DPF; experiments 1–3); four larval stages at 2, 11, 26, and 46 DPH (experiments 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, wherein the fish were treated in an identical manner as the exposed animals except that the electrical field was not switched on.

Survival was monitored until 2 DPH every 5 d for exposed embryos, through 1 week postexposure for larvae, and through 29 d postexposure for juveniles. Morphometric characteristics were measured. Samples for morphometric analysis were taken at two time points for each experiment. The first sampling point was at 2 DPH for embryos and at 1 d postexposure for larvae and juveniles. The second sampling point was at least 15 d postexposure and at most 31 d postexposure. All experiments were approved by the Norwegian Animal Experimental Ethical Committee (Permit ID 5185).

TABLE 1. Overview of the different experiments across three batches and eight developmental stages of Atlantic Cod, indicating when samples were taken for exposure to electric current, survival, and morphometric analysis (DPF = d postfertilization; DPH = d posthatch; asterisk = 50% of embryos hatched at 21 DPF = 0 DPH).

Experiment	Batch	Developmental stage	Exposure	Survival	First morphometric sample point	Second 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	Larva	2 DPH	9 DPH	3 DPH	26 DPH
5	2	Larva	11 DPH	18 DPH	12 DPH	27 DPH
6	2	Larva	26 DPH	33 DPH	27 DPH	58 DPH
7	2	Larva	46 DPH	53 DPH	47 DPH	64 DPH
8	3	Young juvenile	60 DPH	89 DPH	61 DPH	89 DPH

Housing and Rearing

All organisms were cultured according to protocols applied at the NOFIMA Centre for Marine Aquaculture Research (Hansen and Puvanendran 2010; Hansen et al. 2015). The embryos (3,200 fish/L) were housed in 25-L, black cylindroconical tanks. At 2 DPH, larvae (120 fish/L) were transferred to 190-L, green cylindrical tanks 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; dissolved oxygen concentration = 9 mg/L; NH_3 < 0.004 mg/L). Water temperature ranged between 4.0°C and 4.5°C for the embryos and was gradually raised to 10°C for larvae from 5 to 10 DPH. Two milliliters of algae (*Nannochloropsis*; Reed Mariculture, Campbell, California) per day were provided to larvae from 2 to 12 DPH. Rotifers and brine shrimp *Artemia franciscana* nauplii were delivered as live food to larvae at 2–29 DPH and at 25–55 DPH, respectively; prey densities (5–10 rotifers/mL; 1–10 *Artemia*/mL) were increased during rearing. At 38 DPH, larvae that were to be exposed to electric current as juveniles received 15 g of 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 being discontinued (Hansen et al. 2015). Dead embryos were removed from tanks daily, and dead larvae were removed two to three times per week.

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 50-mL vial. In this way, the appropriate number of embryos (15,000) or larvae (3,200) was transferred from the incubator tank to a plastic exposure chamber (33 × 24.5 × 21 cm) that contained 12 L of seawater (Figure 1). Within the chamber, two plate-shaped, stainless-steel electrodes (32 × 23 × 0.4 cm) that conformed to the cross-sectional area of the chamber were fixed in parallel at 24.5 cm apart and were connected to the output of an adjustable laboratory pulse generator (LPG; EPLG bvba, Belgium; Stewart 1972; Henry and Grizzle 2004; Bohl et al. 2010). The LPG (Figure 1) was set to produce a unipolar, square-wave PDC. 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 Setup

Exposure of embryos.—Approximately 15,000 embryos were exposed once at each of three stages: 1 DPF (experiment 1), 5 DPF (experiment 2), or 18 DPF (experiment 3). After exposure, each group of 15,000 embryos was transferred to a new 25-L, cylindroconical incubator (600 embryos/L). In total, three exposure tanks and three 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 d until 23 DPF (i.e., 2 DPH), by which time all embryos had hatched into larvae.

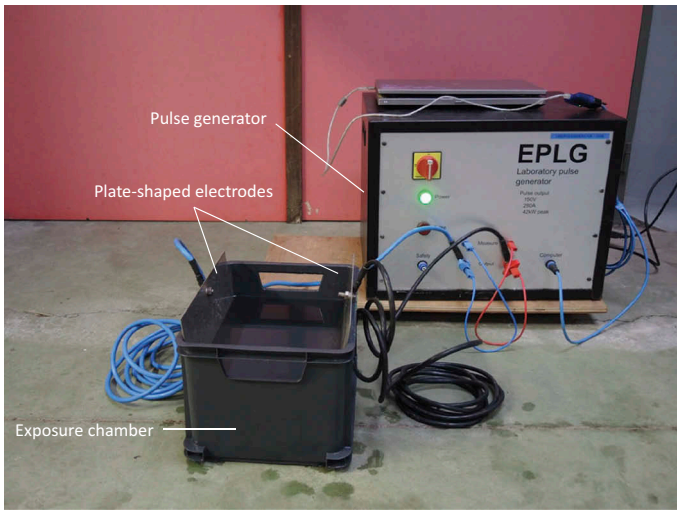

FIGURE 1. Two plate-shaped electrodes connected to the EPLG laboratory pulse generator created a homogeneous electric field to which Atlantic Cod specimens were exposed.

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 per 25 L (120 larvae/L). After hatching, 20 larvae per tank were sampled on a weekly basis through 53 DPH for morphometric analysis described below.

Exposure of larvae.—Electrical pulses were applied to 3,200 larvae at each of four stages: 2 DPH (experiment 4), 11 DPH (experiment 5), 26 DPH (experiment 6), or 46 DPH (experiment 7). After exposure, the animals were maintained in two 25-L, cylindroconical tanks, with two subgroups of exactly 200 larvae in one tank (8 larvae/L) and approximately 3,000 larvae in the other tank (120 larvae/L). In total, 12 tanks (6 exposure tanks and 6 control tanks) were occupied at each developmental stage. At 1 week postexposure, the surviving larvae in the subgroup of 200 larvae were counted and sacrificed. From the subgroup of 3,000 larvae, 20 larvae were sampled for morphometric analysis every week until 58 DPH.

Exposure of juveniles.—At 60 DPH (experiment 8), 200 juveniles were counted, exposed to electric current, and maintained in a 190-L tank (1 juvenile/L). In total, three control tanks and three exposure tanks were occupied by juvenile Atlantic Cod. On a weekly basis, 20 juveniles/tank

were sampled for morphometric analysis and were processed as described below. At 89 DPH, the surviving animals were counted and sacrificed.

Morphometric Analysis

All sampled specimens were euthanized with an overdose of tricaine methanesulfonate (0.7 g/L; 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 from two time points were processed for morphometric analysis as described below. The first time point was 1 DPH for the embryonic stages (experiments 1–3) and 1 d postexposure for the larval and juvenile stages at 3, 12, 27, 47, and 61 DPH (experiments 4–8). For all exposed embryonic stages, the second time point was 22 DPH. Larval stages that were subjected to exposure at 2, 11, 26, and 46 DPH were sampled for morphological analysis at 26, 27, 58, and 64 DPH, respectively (Table 1). The second sampling point for juveniles was 89 DPH. Specimens were photographed by using AnalySIS GetIT software with an Olympus Altra 20 digital camera mounted on an Olympus SZX9 microscope equipped with a 0.5x planar lens (www.olympus.com). Larvae were placed horizontally in a 100-µL droplet of seawater on glass slides, with their left and right palatoquadrate cartilages vertically aligned (Nikolakakis et al. 2014). The following characteristics were measured using ImageJ version 1.46: straight notochord length from the rostral tip of the upper jaw to the caudal tip of the notochord; total notochord length, measured in segments from the tip of the upper jaw along the notochord to its caudal end; eye diameter, measured as vertical eye length; head height, measured through the middle of the eye perpendicular onto the notochord; and muscle height, measured as the vertical length of the notochord muscle near the posterior tip of the gut or anus (Figure 2). The ratio of straight notochord length to total notochord length (incurvation ratio) was calculated as an index of the curvature of larvae and juveniles. Additionally, for the larvae sampled through 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 exposure on the embryo mortality rate was analyzed by use of a mixed model. Replication was set as a random effect; exposure, time, and their interaction were used as categorical fixed effects. The analyses were performed separately for each different exposure timing (i.e., at 1, 5, and 18 DPF). The effects of exposure on the hatching rate at 0 DPH and the mortality rates of larval and juvenile stages were analyzed using a generalized mixed model with a binomial error term (Stroup 2012). Replication was set as a random effect, and developmental stage, exposure, and their interaction were set as categorical fixed effects. The different length measurements were analyzed by using a mixed model with replicate as a random effect and sample time, developmental stage, treatment, and the developmental stage × treatment interaction as fixed effects.

RESULTS

No significant differences in embryo mortality rate were found when exposure to electric pulses took place at 1 DPF ($F_{1, 32} = 0.04$, $P = 0.837$), 5 DPF ($F_{1, 26} = 0.84$, $P = 0.369$), or 18 DPF ($F_{1, 14} = 0.08$, P = 0.776; Figure 3). In all groups, hatching began at 20 DPF. The day by which 50% hatch had occurred (i.e., 0 DPH) was 21 DPF. At the start of the hatching process (at 0 DPH), no significant differences in hatching rates were found between control and exposed groups for embryos exposed at 1 DPF or 5 DPF, but a significant difference was detected for the embryos exposed at 18 DPF (odds ratio =

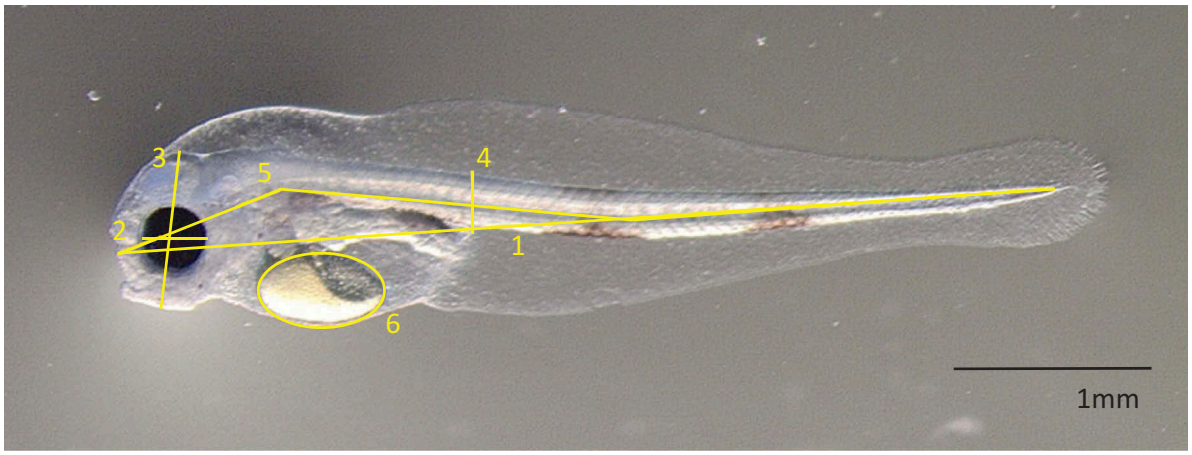

FIGURE 2. Overview of the morphometric measurements taken on early life stages of Atlantic Cod (larva shown here is 1 d posthatch): (1) straight notochord length; (2) eye diameter; (3) head height; (4) muscle height; (5) total notochord length; and (6) yolk surface.

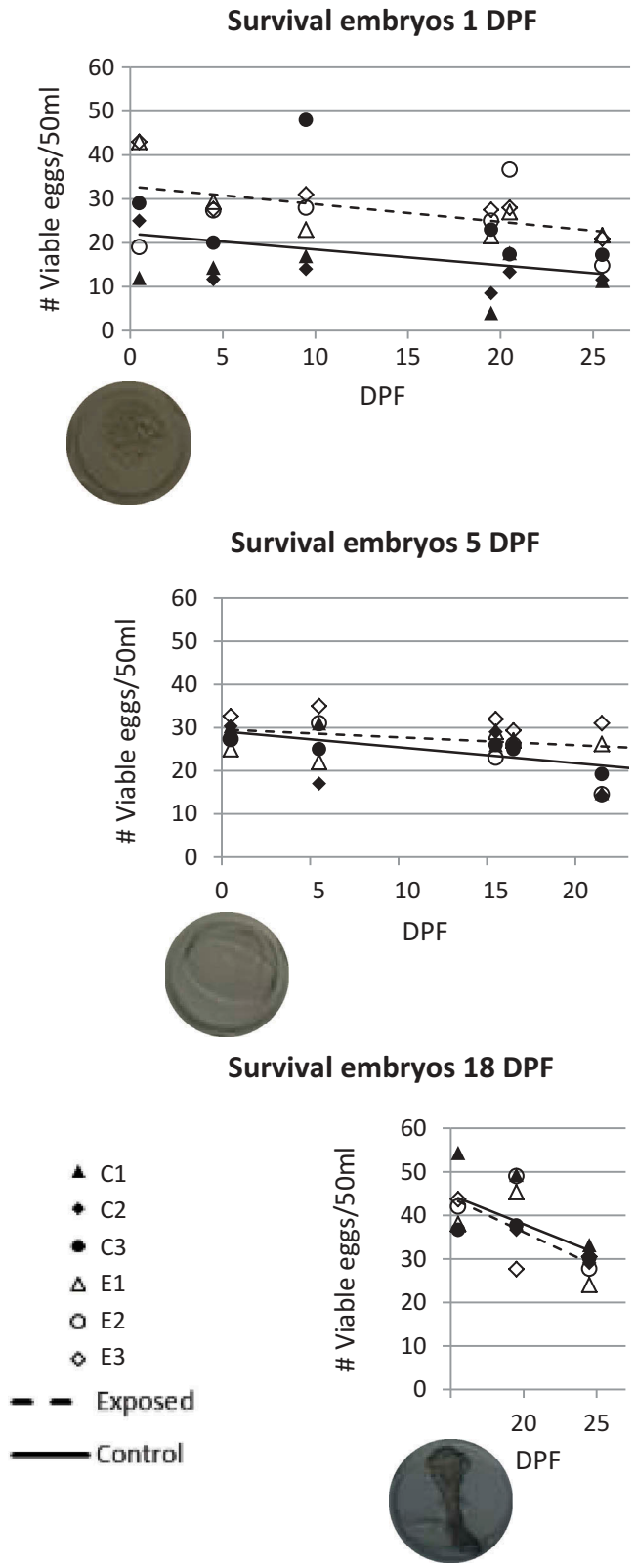

FIGURE 3. Survivorship of Atlantic Cod embryos exposed to electric current at 1, 5, or 18 d postfertilization (DPF).

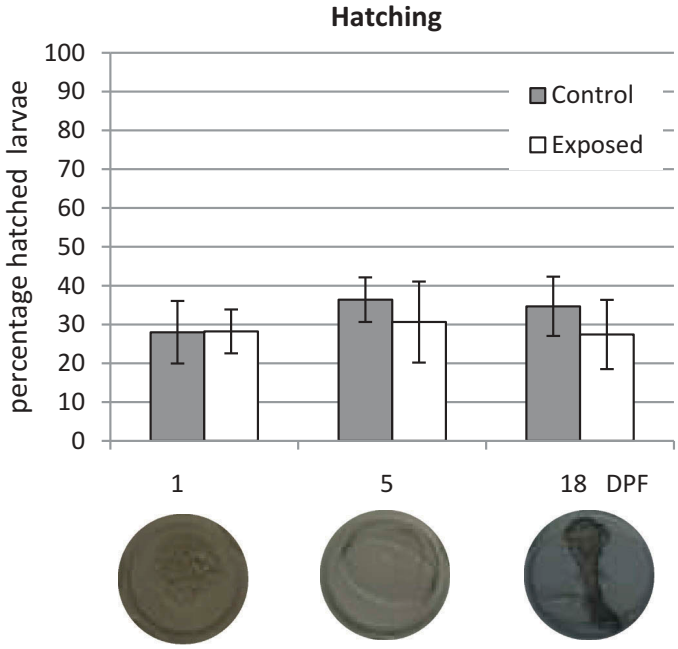

FIGURE 4. Hatching rate at 21 d postfertilization (DPF; 0 d posthatch) for Atlantic Cod embryos that were exposed to electric current at 1, 5, or 18 DPF.

1.43, $P = 0.024$), with a lower initial hatching rate in the exposed group (27%; 95% confidence interval [CI] = 23–32%) than in the control group (35%; 95% CI = 30–40%; Figure 4). However, survival of larvae at 2 DPH did not differ significantly from that of untreated controls ($F_{1, 14} = 0.08$, $P = 0.776$).

In the trials investigating larval survival (Figure 5), the survival of groups exposed at 2, 11, and 26 DPH differed significantly from the survival of control fish, but this was not the case for later exposures at 46 and 60 DPH. At 2 DPH, a lower survival percentage ($P = 0.033$) was observed in the

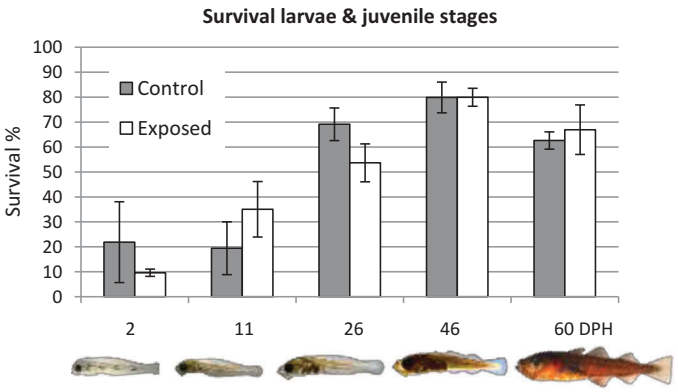

FIGURE 5. Short-term survival rates of Atlantic Cod larvae exposed to an electric field at 2, 11, 26, and 46 d posthatch (DPH) and juveniles exposed at 60 DPH, with data for unexposed controls at the same time points.

exposed group (10%; 95% CI = 8–11%) than in the control group (22%; 95% CI = 10–40%). For exposure at 26 DPH, the difference was even larger (P = 0.001), as survival in the exposed group was equal to 53% (95% CI = 46–61%) while survival in the control group was 69% (95% CI = 63–75%). At 11 DPH, higher survival (P = 0.048) was observed in the exposed group (35%; 95% CI = 25–46%) than in the control group (19%; 95% CI = 11–31%).

No significant differences were detected between control and exposed groups for any of the morphometric measurements (all $P > 0.527$) or for the incurvation ratio ($P = 0.166$) at the first and second sampling points (Supplementary Table S.1).

DISCUSSION

Atlantic Cod embryos were exposed to PDC during early cleavage (1 DPF), epiboly (5 DPF), and near the time of hatching (18 DPF). No differences in mortality rate between exposed and control embryos were found at any of the three egg stages. Reported effects on early life stages, limited primarily to studies of salmonids, are often contradictory (Snyder 2003). Nevertheless, a sufficient number of studies indicates that electrofishing in freshwater over spawning grounds may harm embryos that are present on or in the substrate. Survival was affected, particularly when exposure occurred between the 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 Atlantic Cod embryos in early cleavage are more susceptible to external influences than embryos at later stages because the earlier embryos 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 that are created during electroporation fail to reseal (Chen et al. 2006). Hence, epiboly has been identified as the stage most sensitive to these stressors during development 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. Atlantic 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 et al. 1988), several studies have 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 freshwater species, such as the Largemouth Bass *Micropterus salmoides* and Bluegill *Lepomis macrochirus*, which have comparable egg sizes of approximately 1.7 and 1.1 mm, respectively (Henry and Grizzle 2004). In the present trials, a lower intensity of 1.5 V/cm was applied to the Atlantic Cod eggs in seawater to simulate the shrimp pulse. Furthermore, the type of electrical current applied may be critical to embryo survival. Pulsed DC as used in our experiments has resulted in higher survival than non–pulsed DC (Dwyer and Erdahl 1995; Keefe et al. 2000; Henry and Grizzle 2004).

Electrical fields may induce premature hatching, as was observed in Bluegills and Japanese Medaka *Oryzias latipes*, resulting in an *in situ* increased risk of predation and consequently higher mortality (Yamagami 1988; Henry and Grizzle 2004). In the current study, we observed no higher hatching rate at 0 DPH in exposed groups. In contrast, a lower initial hatching rate was noted for eggs that were exposed at 18 DPF. The reason for this finding is not clear, but it might be attributed to chemical reactions with seawater induced by the electrodes. Indeed, electrolysis of the anode might release metal ions into 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 Atlantic Cod eggs (Grotmol et al. 2003). They may modify the protein polymer compounds in the eggshell, rendering it more resistant to the hatching enzymes that are 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 to 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 only 5-s exposures 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 a speed of 1.54 m/s (3 knots; Stewart 1972). Nevertheless, different chemical reactions might still be possible in the electrically trawled sediment, especially in substrates that are 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 the sporadic muscular contractions that finally cause the chorion to tear (Hall et al. 2004).

The vulnerability of early life stages seems to decrease as development proceeds. However, for some freshwater fish species, the above-mentioned sensitive embryonic stages appear to be less susceptible to electrical stimulation than later posthatch stages (Muth and Rupert 1997; Henry and Grizzle 2003, 2006; Henry et al. 2003). In our experiment, a significantly lower survival rate was noted after exposure of larvae at 2 DPH and 26 DPH in comparison with their corresponding control groups. In the latter stage, many

organ systems are developing (Yin and Blaxter 1987; Pedersen and Falk-Petersen 1992; Brown et al. 2003). Additionally, this stage is more sensitive to external stress because of the transition from cutaneous respiration to gill respiration (Herbing et al. 1996). Larvae were feeding on *Artemia* and needed to chase their prey actively with their yolk completely depleted. In general, this developmental stage is known to be a bottleneck period in Atlantic Cod larviculture conditions, as failure to initiate and maintain sufficient feeding is the major factor contributing to mass mortality (Puvanendran and Brown 1999, 2002; Brown et al. 2003). In contrast, Atlantic Cod at later developmental stages (i.e., 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 the present study, no differences in survival were found for Atlantic Cod exposed during metamorphosis (46 DPH) or at the juvenile stage (60 DPH).

In the present study, a homogeneous electric field of approximately 150 V/m applied for 5 s served as a worst-case scenario of exposure for 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 s based on the assumption that individuals are at rest and only exposed when 150-cm-long electrodes are passing by at a speed of 1.54 m/s. A heterogeneous field implies that field strengths are higher when the electrode is in close proximity (up to 150 V/m at 5 cm) and lower when the distance to the electrode increases (~30 V/m at a moderate distance of 30 cm; Verschueren and Polet 2009; Verschueren et al. 2012). The latter is presumed to be the case for the majority of organisms. Indeed, the potential for Atlantic Cod embryos and larvae to be exposed to an electrical field of 150 V/m during electrofishing will be low because these stages are pelagic and buoyant (Fahay 1983; Markle and Frost 1985), whereas 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, thus increasing their chances for contact with the electrical field (Sundby 1983; Conway et al. 1997). Therefore, young buoyant life stages of Atlantic Cod may have higher chances of contact with the electrical field in the shallow coastal spawning areas (Munk et al. 2002) where shrimp trawling often occurs. Atlantic Cod larvae move to greater depths as they become older (Yin and Blaxter 1987; Heesen and Rijnsdorp 1989) and descend from the water column to bottom habitats at sizes of 2.5–6.0 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 individuals that were exposed during metamorphosis at 46 DPH or during the postmetamorphic juvenile stage at 60 DPH. 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, exposed and control groups did not exhibit differences in the occurrence of 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 (e.g., lordosis and scoliosis).

Eggs were obtained from three batches to ensure that all embryos, larvae, and juveniles used in comparisons were the same age in each experiment to reduce variability in hatching percentage and egg/larval quality between replicates. Indeed, inconsistency in growth rates and survival among tanks is one of the major problems encountered with intensive rearing of larval Atlantic Cod (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 DPH 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 to the findings for larvae exposed at 26 DPH, where the difference between exposed and control groups was much greater.

The present research is innovative in being the first to examine the impact of electrical pulses on a marine fish species during its embryonic, larval, and young juvenile stages, employing the Atlantic 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 on the reproductive success of adults (Cho et al. 2002). Indeed, studies investigating the impacts of electrical pulses on the reproduction of adult broodstock 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 may reduce the viability of subsequently fertilized eggs (Muth and Rupert 1997; Roach 1999). Therefore, a greater proportion of abnormal Atlantic Cod larvae hatching from the 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 *Danio rerio* 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 et al. 2015). In addition, other marine species (e.g., flatfishes) should be included in such studies. Flatfishes demonstrate very complex morphological changes during larval development and metamorphosis, such as migration of the eye (Palazzi et al. 2006; Piccinetti et al. 2012), and might be more vulnerable to

electric pulses. Other species (e.g., herring) that produce demersal eggs should be investigated (Yin and Blaxter 1987), as the eggs could be exposed when electrodes are towed over the seabed.

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