

ARTICLE

Laboratory Study of the Impact of Repetitive Electrical and Mechanical Stimulation on Brown Shrimp *Crangon crangon*

Maarten 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

Bart Verschueren

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

Koen Chiers

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

Luc Duchateau

Department of Comparative Physiology and Biometry, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

Hans Polet

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

Annemie Decostere

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

Abstract

Pulse trawling is currently the best available alternative to beam trawling in the brown shrimp *Crangon crangon* and Sole *Solea solea* (also known as *Solea vulgaris*) fisheries. To evaluate the effect of repetitive exposure to electrical fields, brown shrimp were exposed to the commercial electrodes and pulse settings used to catch brown shrimp (shrimp startle pulse) or Sole (Sole cramp pulse) 20 times in 4 d and monitored for up to 14 d after the first exposure. Survival, egg loss, molting, and the degree of intranuclear bacilliform virus (IBV) infection were evaluated and compared with those in

Subject editor: Isaac Wirgin, New York University School of Medicine, Tuxedo, New York

© Maarten Soetaert, Bart Verschueren, Koen Chiers, Luc Duchateau, Hans Polet, and Annemie Decostere

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

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

Received November 15, 2015; accepted May 16, 2016

stressed but not electrically exposed (procedural control) and nonstressed, nonexposed (control) brown shrimp as well as brown shrimp exposed to mechanical stimuli. The lowest survival at 14 d (57.3%) occurred in the Sole cramp pulse treatment, and this was significantly lower than in the group with the highest survival, the procedural control (70.3%). No effect of electrical stimulation on the severity of IBV infection was found. The lowest percentage of molts occurred in the repetitive mechanical stimulation treatment (14.0%), and this was significantly lower than in the group with the highest percentage of molts, the procedural control (21.7%). Additionally, the mechanically stimulated brown shrimp that died during the experiment had a significantly larger size than the surviving individuals. Finally, no effect of the shrimp startle pulse was found. Therefore, it can be concluded that repetitive exposure to a cramp stimulus and mechanical stimulation may have negative effects on the growth and/or survival of brown shrimp. However, there is no evidence that electrical stimulation during electrotrawls would have a larger negative impact on brown shrimp stocks than mechanical stimulation during conventional beam trawling.

In beam trawl fisheries, tickler chains, chain matrices, or bobbin ropes are used to mechanically stimulate and catch flatfish and brown shrimp *Crangon crangon*. However, these gears have well-known disadvantages, such as high fuel consumption (Poos et al. 2013) and seabed disturbance (Lindeboom and de Groot 1998), resulting from their intense bottom contact (Depestele et al. 2016). Another important disadvantage is that beam trawling is a mixed fishery with poor selectivity, which results in high bycatch rates (Kaiser and Spencer 1995; Depestele et al. 2014; Bayse et al. 2016). Most of these mainly undersized fish and nonmarketable species are subsequently discarded. In the reformed Common Fisheries Policy, the European Commission has selected beam trawling as one of the first fisheries to implement the discard ban and further stated that unwanted bycatch should be reduced in this fishery (European Council 2012).

The most promising alternative to beam trawling is pulse fishing, in which mechanical arousal by tickler chains or bobbins is replaced by electrical stimulation with electrodes, inducing electrical pulses. The use of electricity in these so-called electrotrawls to catch marine organisms was prohibited by the European Commission in 1988 (European Council 1998). Nevertheless, in 2009 European Union member states were granted an exemption permitting 5% of the fleet to use pulse trawls in the southern North Sea, which was extended to 10% of the fleet in early 2014. By January 2016, 91 vessels had already adopted this technique commercially, of which 1, 3, 10, and 77 have Belgian, UK, German, and Dutch licenses, respectively (Soetaert et al. 2016). Although these vessels differ in rigging and the weight of fishing gear, their electrical parameters are similar and can be roughly divided into two pulse types as a function of the target species. The first type, constituting the vast majority of pulse vessels, targets flatfish, particularly Sole *Solea solea* (also known as *Solea vulgaris*). These electrotrawls use a bipolar cramp pulse with a frequency of around 80 Hz that elicits a cramp reaction in the fish's muscles which immobilizes it and causes it to bend into a U-shape (Soetaert et al. 2016). As a consequence, Sole cannot flee and are more easily scooped up by the foot rope of the fishing gear, which increases catch efficiency (Soetaert et al. 2015). In these gears, tickler chains are replaced by

electrodes consisting of a series of isolated and conductive parts connected to the beam trawl or its alternative (sumwing, sewing, or multiwing) and towed over the seabed, followed by a footrope (van Marlen et al. 2014). The removal of tickler chains 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). A minority of the electrotrawls target brown shrimp by producing a unipolar startle pulse of 5 Hz. This pulse induces five contractions of the brown shrimp's abdomen per second, each time resulting in a tail flip (Polet et al. 2005a). As a consequence, the brown shrimp jump out of the sediment into the water column, while small flatfish, most other benthos, and debris are not stimulated and remain on or close to the seafloor (Polet et al. 2005b). This allows fishermen to use a straight bobbin rope with 10–12 (instead of 36) bobbins and a higher footrope, which can reduce bycatch volumes up to 75% (Verschueren et al. 2014). However, before a general exemption on this fishery can be implemented, several concerns about the negative effects on target and nontarget species need to be addressed (ICES 2009).

One of the main concerns is the possible negative impact of the electrical pulses on invertebrates, as these are most often not caught and can be exposed repetitively. Two exploratory reports evaluated the behavior and survival of invertebrates exposed to the cramp pulse for Sole near wire-shaped electrodes. Smaal and Brummelhuis (2005) exposed on average 10 individuals of 19 species of molluscs, echinoderms, crustaceans, and polychaetes to electrical pulses with an amplitude twice as high and an exposure time eight times as long as the settings used on commercial vessels targeting Sole. The reactions during exposure were minor or negligible, and survival after 3 weeks did not differ from that of the control group. Van Marlen et al. (2009) exposed a selection of six benthic invertebrates to three subsequent bursts of 1 s in duration. For each species, they exposed 20 animals at three different distances, ranging from 0.1 to 0.4 m from the electrode. Compared with the control groups, there were significant reductions in the survival rate of exposed king ragworm *Allitta virens* and European green crab *Carcinus maenas* of 3% and 5%, respectively, when all exposures were clustered by species regardless of the distance from the electrode. Atlantic razor clams *Ensis directus* exhibited a significant 7% reduction in

survival rate at 0.1 m from the electrodes but a greater survival rate at 0.2 m. The latter result is odd, since Murray et al. (2016) did not find an impact on the survival of the razor clam *Ensis siliqua* exposed in both laboratory and field trials to nonpulsed alternating current. Furthermore, food intake was significantly reduced by 10–13% in the European green crab. No significant effects were found for common prawns *Palaemon serratus*, surf clams *Spisula solidissima*, and common starfish *Asterias rubens*. As a result, it was concluded that the electrical pulses used to catch Sole are less invasive than the effects of conventional beam trawling with mechanical stimulation. However, both studies examined only the effect of the cramp stimulus for Sole, and the variable results obtained by van Marlen et al. (2009) suggest that not enough animals were included to exclude the variability due to natural mortality. Therefore, the results in these reports should be interpreted with caution and more extensive survival studies are needed.

To meet these concerns, Soetaert et al. (2014) recommended evaluating the survival, gross lesions, and microscopic lesions of large numbers of king ragworm and brown shrimp 14 d after exposure to various electrical pulses in a homogeneous electrical field with plate-shaped electrodes. Exposure of animals to a single electrical pulse with varying pulse parameters did not result in increased mortality or more lesions (Soetaert et al. 2014). However, brown shrimp exposed to the highest electrical field strength showed an increase in the number and size of intranuclear bacilliform virus (IBV) infection in the hepatopancreas. In addition, no discernible negative effects were found 14 d after four repetitive exposures to the shrimp startle pulse or Sole cramp pulse (Soetaert et al. 2014). However, side effects could not be completely ruled out for this important commercial species. Therefore, it was argued that additional experiments were warranted to evaluate the impact of repetitive exposure to the commercial, wire-shaped electrodes and pulses that may be used in actual fishing practice.

The purpose of the present study was to evaluate the effects on brown shrimp of repeated exposure to the startle and cramp pulses used in the field by commercial electrotrawls targeting brown shrimp and Sole, respectively, and to compare these effects with those of conventional mechanical stimulation. In contrast to previous controlled laboratory studies, the commercial electrodes and pulse settings were selected to mimic the situation in the field as closely as possible. Survival, molting, and macroscopic and microscopic lesions were quantified. Additionally, gravid female brown shrimp were included in the study to determine potential egg loss following exposure.

METHODS

Animals and housing facilities.—In total, 1,079 brown shrimp with a mean \pm SD exoskeleton length of 64.4 ± 6.5 mm were included. The use of these large animals allowed for an evaluation of the impacts of electrical and mechanical stimulation on egg retention. The animals were

caught along the Belgian coast with a commercial 4-m brown shrimp beam trawl and transported to the housing facilities within 3 h. There they were allowed to acclimate for 5 d, being housed and fed as described by Soetaert et al. (2014). The shrimp were randomly divided into 18 experimental tanks ($0.75 \times 0.55 \times 0.30$ m), each containing 58–60 animals (38–41 with eggs and 19–20 without eggs). The water parameters were as follows: 12°C temperature; 35‰ salinity; 4.29 S/m conductivity; pH 8.0; 6°KH carbonate water hardness; <25 mg/L nitrate; <0.2 mg/L nitrite; and <0.1 mg/L ammonia.

Electrical setup.—All pulses were generated by a laboratory pulse generator (LPG; EPLG, Bruges, Belgium) with a maximum peak output of 150 V, 280 A, and 42 kW. Wire-shaped electrodes such as those used in the commercial fishery were adapted as described in Soetaert et al. (2015). For the shrimp startle pulse, two electrodes of 0.5-m length were placed 0.6 m apart (measured from the center; Figure 1A). A 5-Hz pulsed direct current with a pulse duration of 0.5 ms was applied. For the Sole cramp pulse, two 0.18-m conductors were placed at a distance of 0.42 m from the center of the tank (Figure 1B). An 80-Hz pulsed bipolar current with a pulse duration of 0.25 ms was applied. All electrodes were mounted in PVC netting material to guarantee a fixed and reproducible mutual distance as well as a vertical distance of 10 mm above the bottom of the tank to simulate a more natural field distribution. In both setups, the duration of each exposure was 1 s and a potential difference of 60 V was used. The pulse shapes were simulated, including an inductive effect, to accurately mimic the field situation.

Experimental design.—Five experimental treatments were used: no stressor (CTRL; 243 animals over four replicates), 20 exposures to the shrimp startle pulse (SHRI; 241 animals over four replicates), 20 exposures to the Sole cramp pulse (SOLE; 238 animals over four replicates), a procedural control with 20 exposures to alternating shrimp and Sole electrodes without an electrical stimulus (ELEC; 179 animals over three replicates), and 20 exposures to a mechanical stimulus (MECH; 178 animals over three replicates). Immediately prior to the first exposure, 30 brown shrimp (15 with and 15 without eggs) were randomly selected, sacrificed, and processed for histological analysis as described by Soetaert et al. (2014), with special attention to the epithelium of the cardiac stomach, the hepatopancreas, the heart, and the caudal muscles. Furthermore, the severity of an intranuclear bacilliform virus infection in the hepatopancreas was examined and an average score was given as described in Soetaert et al. (2014).

The brown shrimp in the SHRI and SOLE groups were exposed to electrical pulses as described above. Prior to each exposure, the cover of the tank was removed and the electrodes were gently inserted into the water to minimize the disturbance to the animals in the tank. Ten seconds later, the animals were exposed. After the brown shrimp had resettled into the sediment, the electrodes were gently removed and the tank was covered again. The brown shrimp in the procedural

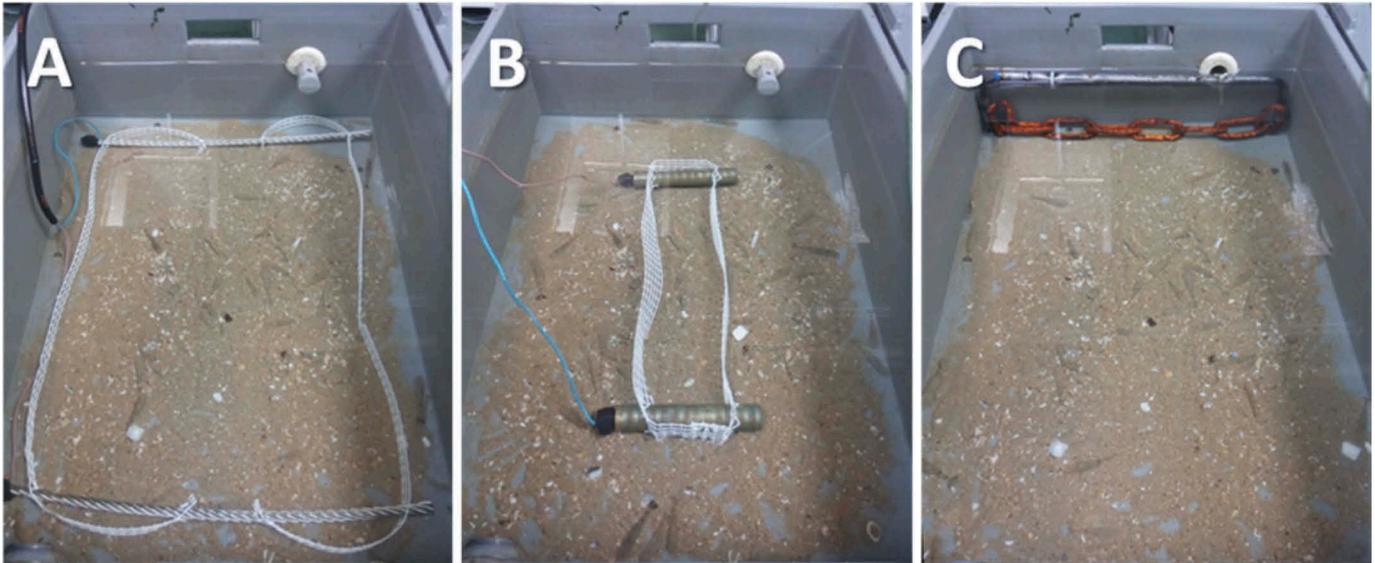


FIGURE 1. Experimental setups used for (A) the shrimp startle pulse, (B) the Sole cramp pulse, and (C) mechanical chain stimulation. For the electrical stimulation of the shrimp (A and B), powerleads were placed in the tank from the left and connected to the ends of the electrodes, which were 1 cm above the bottom of the tank by means of white PVC netting strips. The mechanical stimulation (C) was carried out using a chain mounted on a U-shaped grip that was pulled through the tank at approximately 1 m/s. The tanks ($0.75 \times 0.55 \times 0.30$ m) had a water depth of 0.2 m.

control (ELEC) were treated the same way as those in the SHRI and SOLE groups, but no electrical stimulus was applied. Those in the MECH group were mechanically stimulated using a chain mounted on a U-shaped grip that was pulled at approximately 1 m/s through the tank (Figure 1C). All stimuli were applied five times a day at 90-min intervals over four successive days (i.e., one fishing week), after which the brown shrimp were monitored for another 10 d. Dead individuals and molts were removed daily. The number and exoskeleton size of the dead brown shrimp were recorded separately for individuals with and without eggs. Fourteen days after the first exposure (DPFE), the number of surviving brown shrimp with and without eggs was determined for each tank. Three with and three without eggs from each replicate were randomly selected, sacrificed, measured, and processed for histological examination as described above. The percentage of molts was defined as the ratio between the number of molts and the number of animals initially stocked in the tanks, times 100. The percentage egg loss was calculated as

$$100 \cdot [1 - (DCE + SCE)/TCE],$$

where DCE is the total number of shrimp that died during the 14-d monitoring period that still carried eggs, SCE is the number of shrimp that survived until 14 DPFE that still carried eggs, and TCE is the total number of shrimp that carried eggs at the start of the experiment.

Statistics.—The statistical analysis investigated differences in survival between treatments at 7 and 14 DPFE, egg loss at 14 DPFE, and the percentage of molts at 14 DPFE using the

generalized mixed model with a binomially distributed error term and replication as a random effect. At each time, nine pairwise comparisons were made (CTRL versus MECH, SHRI, and SOLE; ELEC versus MECH, SHRI, and SOLE; MECH versus SHRI and SOLE; and SHRI versus SOLE); the comparisonwise level for significance was set at $0.05/9 = 0.0056$. The difference between the sizes of dead and surviving brown shrimp at 14 DPFE was based on the fixed-effects model and was done separately for each treatment. The sizes of the brown shrimp that died were also compared among the different treatments using the fixed-effects model. The effect of treatment on the IBV-score was based on the Kruskal–Wallis test, and the correlation with 7 and 14 DPFE mortality was determined using Kendall's correlation coefficients.

RESULTS

Brown shrimp exposed to the brown shrimp startle pulse (SHRI group) showed startle behavior, while those subjected to the Sole cramp pulse (SOLE group) displayed a cramp reaction, both followed by an escape response as described by Soetaert et al. (2014). During this escape response the shrimp jumped in random directions for 1–3 s, whereafter they resettled and reburied themselves in the sediment. However, the brown shrimp in the SOLE group located at the corners of the tank demonstrated less intensive cramp behavior, with attenuated tail flipping, than the animals situated in the center. Animals exposed to the mechanical stimulus (MECH group) either immediately reburied themselves or exhibited a short escape reaction. Brown shrimp from the

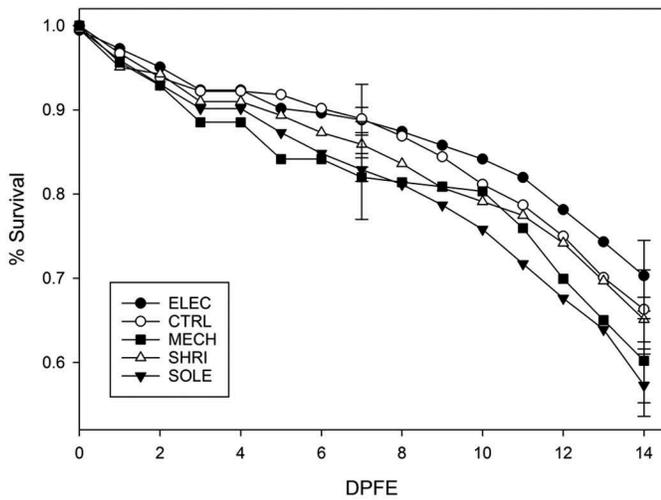


FIGURE 2. Average survival rates of brown shrimp in five different treatments as a function of DPFE. Error bars (SEs) are given only for 7 and 14 DPFE to avoid crowding.

CTRL and ELEC groups did not display a tail flipping reaction, and all animals remained buried in the sand except when accidentally touched.

The amount of food consumption declined gradually over time, with food being leftover from 8 DPFE onward. Similarly, the number of animals immediately starting to feed decreased continuously during the 14-d monitoring period. This was accompanied by a gradual decrease in the number of surviving individuals in all experimental treatments, as shown in Figure 2. The percentages of animals that survived, molted, and/or lost eggs are given in Table 1. Significantly lower survival at 14 DPFE was found for the SOLE group than for the procedural control group (ELEC) ($P = 0.0034$), as was a significantly lower percentage of molts for the MECH group than for the ELEC group ($P < 0.0001$). No differences were found in the sizes of the brown shrimp that died (Table 2), but the size of the surviving shrimp in the MECH group was significantly smaller than that of those that had died ($P = 0.0175$; $\alpha = 0.05$).

TABLE 2. Mean \pm SE sizes of brown shrimp that died by 14 DPFE, sizes of those that survived at 14 DPFE, and severity of intranuclear bacilliform virus (IBV) infections among surviving shrimp at 14 DPFE for five experimental groups (see Table 1). The average score for IBV prior to exposure was 1.54 ± 0.22 . The only significant difference among these comparisons was between the sizes of dead and surviving shrimp in the MECH treatment.

Group	Size (mm)		IBV score
	Dead	Surviving	
CTRL	65.0 \pm 0.8	64.1 \pm 0.5	1.88 \pm 0.19
ELEC	66.1 \pm 0.9	64.1 \pm 0.6	1.11 \pm 0.29
MECH	65.2 \pm 0.8	62.8 \pm 0.6	1.33 \pm 0.26
SHRI	64.6 \pm 0.7	63.9 \pm 0.5	1.17 \pm 0.21
SOLE	66.1 \pm 0.7	64.4 \pm 0.6	1.21 \pm 0.20

Histological examination did not reveal acute or subacute lesions such as bleeding, inflammation, loss of tissue integrity, or cell mortality. However, in 5% of the individuals examined in all groups, intramuscular nematodes were present. The mean \pm SE IBV score prior to exposure was 1.54 ± 0.22 . This increased in the CTRL group during the 14-d experiment but decreased in all other treatments (Table 2). However, statistical analysis did not reveal significant differences between the IBV scores of the different groups. In addition, no correlation was found between mortality and the IBV score.

DISCUSSION

In the present study, the experimental setup was designed to mimic commercial electrotrawling for Sole as closely as possible by using commercial, wire-shaped electrodes to generate a heterogeneous electrical field. This setup resulted in greater variation in the electrical doses experienced by the animals—in contrast to a previous study in which a homogeneous setup with plate-shaped electrodes was employed (Soetaert et al. 2014). Indeed, the intensity of the electrical field decreased exponentially with the distance to wire-shaped electrodes. De

TABLE 1. Mean \pm SE percentages of brown shrimp surviving to 7 and 14 d after first exposure to an electrical or mechanical stimulus (DPFE) and percentages that had molted or lost eggs at 14 DPFE. Groups are as follows: CTRL = not exposed, ELEC = exposed to electrodes without any pulse, MECH = exposed to mechanical stimulation by means of a towed chain, SHRI = exposed to pulses generated by a commercial setup to catch brown shrimp, and SOLE = exposed to pulses generated by a commercial setup to catch Sole. Within columns, different lowercase letters denote significant differences between values.

Group	Survival		Molts	Egg loss
	7 DPFE	14 DPFE		
CTRL	88.9 \pm 4.1 z	66.3 \pm 4.7 zy	18.1 \pm 2.0 zy	30.6 \pm 5.4 z
ELEC	88.8 \pm 1.5 z	70.3 \pm 4.2 z	21.7 \pm 1.4 z	29.3 \pm 5.4 z
MECH	82.0 \pm 5.0 z	60.1 \pm 5.0 zy	14.0 \pm 2.2 y	25.6 \pm 9.0 z
SHRI	85.9 \pm 2.7 z	65.1 \pm 2.6 zy	18.7 \pm 3.3 zy	31.7 \pm 1.9 z
SOLE	82.9 \pm 1.4 z	57.3 \pm 3.7 y	18.3 \pm 2.3 zy	31.2 \pm 4.5 z

Haan et al. (2011) conducted field strength measurements around the same electrodes as used in the present study. A potential difference of 50–60 V resulted in field strengths that varied between 70 and 400 V/m when measured 20 mm above the tank floor in the water between the electrodes and between 20 and 50 V/m in the 0.15 m around the electrodes when measured 80 mm above the tank floor. Based on the measurements and simulations of Verschueren et al. (2014), the minimal field strength above the tank floor in the SHRI setup was around 50 V/m and the maximum around 400 V/m. This variability in field strength, inherent in a setup with wire-shaped electrodes, was reflected in the less pronounced cramp reaction of the animals situated at the corners of the tanks. This observation suggests that the electrical field of commercial electrotrawls decreases rapidly beyond the trawl and that no effective stimulation outside the trawl path is to be expected (Polet et al. 2005a; De Haan et al. 2011). Although the intensity of the single electrical exposures experienced by the animals was variable, the total impact on the population of shrimp in the tank was averaged across a large number of exposures and animals as well as the random redistributions of the shrimp during their escape responses after every exposure. Furthermore, the electrical setups in the present study resulted in greater electrical field strengths than those encountered in actual fisheries. In the SHRI setup this resulted from the reduced distance between the electrodes, which was limited to 0.6 m due to tank size limitations, compared with the 0.7 m used in the field. In the SOLE exposures, a 60 V potential difference on the electrodes was applied, which is higher than the 50–55 V used in electrotrawls (Soetaert et al. 2015). Additionally, the sequence of exposures (20 times in one fishing week of 4 d) is most likely more frequent than would occur in the field, where only 0.6% of the seabed is estimated to be trawled more than 20 times a year (Rijnsdorp et al. 1998). Therefore, we believe that the present experimental setup most likely represents a worst-case scenario with respect to exposures to electrotrawl pulses in the field.

The effect on survival differed depending on the pulse used. The lowest survival at 14 DPFE occurred in the SOLE group exposed to 80-Hz pulses ($57.3 \pm 3.7\%$), which was significantly lower than that for the ELEC group, which had the highest survival ($70.3 \pm 4.2\%$). No significant differences were found with the 5-Hz pulses used to startle shrimp (SHRI), suggesting that there were no negative effects of this stimulus on brown shrimp. This agrees with the results obtained by Soetaert et al. (2014) in a homogeneous setup and with exposure studies with fish. Desender et al. (2016) exposed European Plaice (also known as Plaice) *Pleuronectes platessa*, Sole, Atlantic Cod *Gadus morhua*, Shorthorn Sculpin *Myoxocephalus scorpius*, and Armed Bullhead *Agonus cataphractus* to the same electrical stimulus applied by a similar electrode setup for 5 s, but no or only minor and reversible effects were reported. The difference may be explained by the electrical load, as the duty cycle

(the portion of time that the electric current is effectively running), was 8 times as long for the SOLE group as for the SHRI group. This effect was not observed in a previous study using the same pulse in a homogeneous setup with more intense but less frequent exposures (Soetaert et al. 2014). Possibly, the quite high 14-d mortality of 30–43% as well as the relatively high standard errors (2.6–5%) (Table 1) interfered with the results. This might explain the lower survival of the CTRL group relative to that of the procedural control (ELEC). This issue in survival experiments with brown shrimp was previously revealed by Verhaegen (2012) and was also observed by Soetaert et al. (2014). Therefore, it remains to be elucidated whether this represents a consistent finding. In further survival experiments with brown shrimp, increasing the number of individuals tested is recommended.

In a previous study, an increased IBV infection rate was found in the hepatopancreas of brown shrimp exposed to field strengths of 200 V/m in a homogeneous setup, indicating a possible indirect effect of exposure to electrical pulses (Soetaert et al. 2014). In the present study, by contrast, such an increase was not observed despite the repetitive exposures. Rather, the highest IBV infection rate was found in the non-stressed control group. We speculate that the brown shrimp with the highest IBV loads died, and since the IBV score was determined from surviving animals this resulted in a lower mean. However, the lack of correlation between the severity of IBV infections and mortality casts doubt on this hypothesis. Another reason may be that in the heterogeneous setup that we employed, field strengths of 200 V/m or higher were only present in close proximity to the conductors' center (De Haan et al. 2011). As a consequence, this high field strength was only experienced by a minority of the brown shrimp and only for a very short time, as they immediately jumped out of this range. Therefore, the number of brown shrimp exposed to sufficiently strong and long pulses may be too low in the commercial electrotrawl setup to obtain higher observations of IBV infection.

The percentages of molts and egg loss were monitored in the present experiments. This study is the first to employ these variables to discern possible sublethal effects of repeated exposures and to investigate whether electrotrawling can interfere with reproduction. No significant effect of electrical stimulation on egg loss was demonstrated, although partial egg loss may have been unnoticed. Similarly, electrical stimulation did not affect the percentage of molts compared with mechanical stimulation, but the MECH group showed the lowest percentage of molts, significantly lower than the ELEC group. In addition, the brown shrimp in the MECH treatment that died during the experiment had a significantly larger size than the surviving individuals, which was not observed in other treatments. We hypothesize that larger shrimp have a higher probability of being impacted or crushed by the chain, either during passage or when arriving at the end of the tank. This may result in a higher mortality of large shrimp, resulting

in a smaller average size for the surviving individuals. Indeed, physical injuries have been shown to affect survival adversely (Bergmann et al. 2001; Depestele et al. 2014), which may be caused by damage to their fragile exteriors (Kaiser and Spencer 1995). Moreover, injuries demand extra expenditures of energy with subsequent decreases in growth and molt increments (Bennett 1973), which would explain the difference in the percentage of molts encountered.

The latter findings led us to speculate on the actual significance of the negative impact of repetitive exposures to the Sole cramp pulse in brown shrimp in commercial fishing practice. Brown shrimp are often encountered on fishing grounds in which beam trawls target Sole. If repetitive exposure to the Sole cramp pulse has a larger negative impact on the survival and growth of brown shrimp than conventional mechanical stimulation, a conversion to pulse stimulation would pose a greater threat to the stocks of brown shrimp and the commercial fishery for them. This was a major concern of fishermen targeting brown shrimp and the motive for the present study. Therefore, any detrimental effects of the Sole cramp pulse should be balanced against the larger mechanical impact of conventional trawling targeting Sole. The SOLE and the MECH groups were significantly affected (compared with the procedural control) in terms of survival and molting, respectively, which argues for caution. However, no significant differences were found between the SOLE and MECH groups, indicating that an increased negative impact of the electrical stimuli is unlikely. Indeed, no adverse effects from the exposure of brown shrimp to a startle pulse have been observed in the present or previous studies (Polet et al. 2005a; Soetaert et al. 2014). Because the use of this stimulus enables electrotrawls targeting brown shrimp to reduce their bycatch rates of juvenile small shrimp (Verschuere et al. 2014), their impact on brown shrimp stocks will most likely be smaller than that of conventional beam trawls if landings are not increased.

In conclusion, brown shrimp exposed to the stronger Sole cramp pulse had the lowest survival, which was significantly different from that of those exposed to electrodes without electrical pulses. However, no differences in mortality were noted with brown shrimp that were exposed to mechanical stimulation or with those that were not stressed at all or exposed to the shrimp startle pulse. The repetitive mechanical stimulation of adult brown shrimp by a tickler chain resulted in a decreased percentage of molts and in size-specific mortality, with larger shrimp experiencing reduced survival. Extrapolating the present results to commercial fishing practice, it can be concluded that the use of electrotrawls to target brown shrimp is likely to be less detrimental than conventional beam trawls. The results for the cramp pulse used by electrotrawls targeting Sole are more ambiguous, but even with very frequent exposures the impact of electrical stimulation was no larger than that of mechanical stimulation.

ACKNOWLEDGMENTS

Maarten Soetaert's research was supported by a Ph.D. grant from the Institute for the Promotion of Innovation by Science and Technology in Flanders. Additional support was provided by the Flemish Scientific Institute for Agricultural and Fisheries Research and the European Fisheries Fund. We thank Christian Puttevels and Delphine Ameys for embedding and processing the samples and P. Simoens for critically reviewing the manuscript.

REFERENCES

- Bayse, M. S., B. Herrmann, H. Lenoir, J. Depestele, H. Polet, E. Vanderperren, and B. Verschuere. 2016. Could a T90 mesh cod end improve selectivity in the Belgian beam trawl fishery? *Fisheries Research* 174:201–209.
- Bennett, D. B. 1973. The effect of limb loss and regeneration on the growth of the edible crab, *Cancer pagarus*. *Journal of Experimental Marine Biology and Ecology* 13:45–53.
- Bergmann, M., D. J. Beare, and P. G. Moore. 2001. Damage sustained by epibenthic invertebrates discarded in the *Nephrops* fishery of the Clyde Sea area, Scotland. *Journal of Sea Research* 45:105–118.
- De Haan, D., J. E. Fosseidengen, P. G. Fjellidal, 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.
- Depestele, J., I. Ana, K. Degrendele, M. Esmaceli, 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.
- Depestele, J., M. Desender, H. P. Benoit, H. Polet, and M. Vincx. 2014. Short-term survival of discarded target fish and nontarget invertebrate species in the "eurocutter" beam trawl fishery of the southern North Sea. *Fisheries Research* 154:82–92.
- Desender, M., K. Chiers, H. Polet, B. Verschuere, J. Saunders, A. Mortensen, V. Puvanendran, and A. Decostere. 2016. Electrotrawling for brown shrimp: short-term effects on various adult fish species. *Fisheries Research* 179:90–97.
- European Council. 1998. Regulation 850/98 for the conservation of fishery resources through technical measures for the protection of juveniles of marine organisms. Official Journal of the European Union L 125.
- European Council. 2012. Proposal for a regulation of the European Parliament and of the Council on the European Maritime and Fisheries Fund [repealing Council Regulation (EC) No. 1198/2006 and Council Regulation (EC) No. 861/2006 and Council Regulation No. 1255/2011 on integrated maritime policy. European Council, Brussels.
- ICES (International Council for the Exploration of the Sea). 2009. 1.5.6.2 Answer to Dutch request on electric pulse trawl. Pages 157–165 in Report of the ICES Advisory Committee, 2009. Book 1: introduction, overviews, and special requests. ICES, Copenhagen.
- Kaiser, M. J., and B. E. Spencer. 1995. Survival of bycatch from a beam trawl. *Marine Ecology Progress Series* 126:31–38.
- Lindeboom, H. J., and S. J. de Groot. 1998. Impact II: the effects of different types of fisheries on the North Sea and Irish Sea benthic ecosystem. International Council for the Exploration of the Sea, Document C003/98, Copenhagen.
- Murray, F., P. Copland, P. Boulcott, M. Robertson, and N. Bailey. 2016. Impacts of electrofishing for razor clam (*Ensis* spp.) on benthic fauna. *Fisheries Research* 174:40–46.
- 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.
- Poos, J. J., M. N. J. Turenhout, H. A. E. van Oostenbrugge, and A. D. Rijnsdorp. 2013. Adaptive response of beam trawl fishers to rising fuel cost. *ICES Journal of Marine Science* 70:675–684.
- Rijnsdorp, A. D., A. M. Buys, F. Storbeck, and E. G. Visser. 1998. Microscale distribution of beam trawl effort in the southern North Sea between 1993 and 1996 in relation to the trawling frequency of the seabed and the impact on benthic organisms. *ICES Journal of Marine Science* 55:403–419.
- Smaal, A. C., and E. Brummelhuis. 2005. Onderzoek naar mogelijke effecten van de pulskor op bodemdieren. [Research on possible effects of pulse trawling on benthic organisms.] International Council for the Exploration of the Sea, Document C089/05, Copenhagen.
- Soetaert, M., K. Chiers, L. Duchateau, H. Polet, B. Verschuere, 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. Verschuere, and K. Chiers. 2015. Electrotrawling: a promising alternative fishing technique warranting further exploration. *Fish and Fisheries* 16:104–124.
- Soetaert, M., A. Decostere, B. Verschuere, 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* 177:95–103.
- van Marlen, B., D. De Haan, A. van Gool, and D. Burggraaf. 2009. The effect of pulse stimulation on marine biota: research in relation to ICES advice—progress report on the effects on benthic invertebrates. International Council for the Exploration of the Sea, Document C103/09, Copenhagen.
- 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.
- Verhaegen, Y. 2012. Mode of action, concentration, and effects of tributyltin in common shrimp *Crangon crangon* L. Doctoral dissertation. Ghent University, Ghent, Belgium.
- Verschuere, B., H. Lenoir, L. Vandamme, and B. Vanellander. 2014. Evaluatie van een seizoen pulsvisserij op garnaal met HA31. [Pulse trawling for brown shrimp onboard the HA31: a year-round evaluation.] International Council for the Exploration of the Sea, Document 157, Copenhagen.