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Determining the safety range of electrical pulses for two benthic invertebrates: brown shrimp (*Crangon crangon* L.) and ragworm (*Alitta virens* S.)

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Pulse trawling is currently the most promising alternative for conventional beam trawls targeting sole and shrimp, meeting both the fisher's aspirations and the need for more environmentally friendly fishing techniques. Before electrotrawling can be further developed and implemented on a wider scale, however, more information is needed about the effects of electrical pulses on marine organisms. The organisms used in the present experiments were brown shrimp (*Crangon crangon* L.) and king ragworm (*Alita virens* S.) as model species for crustaceans and polychaetes, respectively. These animals were exposed to a homogeneously distributed electrical field with varying values of the following parameters: frequency (5–200 Hz), electrical field strength (150–200 V m^{-1}), pulse polarity, pulse shape, pulse duration (0.25–1 ms), and exposure time (1–5 s). The goal of this study was to determine the range of safe pulses and thereby also to evaluate the effect of the pulses already being used on commercial electrotrawls. Behaviour during and shortly after exposure, 14-d mortality rates, and gross and histological examination were used to evaluate possible effects. The vast majority of shrimp demonstrated a tail flip response when exposed to electric pulses depending on the frequency, whereas ragworm demonstrated a squirming reaction, independent of the frequency. No significant increase in mortality or injuries was encountered for either species within the range of pulse parameters tested. Examination of the hepatopancreas of shrimp exposed to 200 V m^{-1} revealed a significantly higher severity of an intranuclear baculoform virus infection. These data reveal a lack of irreversible lesions in ragworm and shrimp as a direct consequence of exposure to electric pulses administered in the laboratory. Despite these promising results, other indirect effects cannot be ruled out and further research hence is warranted.

Keywords: benthic invertebrates, effects, electrical pulses, electrotrawling, histology, injuries, pulse trawling, survival.

Introduction

In traditional beam trawl fishery, tickler chains, chain mats, or bobbin ropes are used to target flatfish or shrimp. These fishing gears are usually heavy and have a high drag, resulting in the well-known disadvantages including high fuel consumption and seabed disturbance. Another important disadvantage of beam trawling is its poor selectivity. This mixed fishery targets several species with highly varied minimum landing sizes, which results in bycatch. Most of these mainly undersized fish and non-marketable species are subsequently discarded. In the reformed Common Fisheries Policy (CFP), the European Commission has selected beam trawling as one of the first fisheries to implement

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the discard ban and further stated that unwanted bycatch should be reduced in this fishery (Council of the European Union, 2012).

Pulse trawling seems to be the most promising alternative for conventional beam trawling. In these electrotrawls, the mechanical stimulation by tickler chains, chain mats, or bobbins is (partly) replaced by electrical stimulation. These electrodes are hanging on the beam and tow over the seabed, followed by a footrope or straight bobbin rope with a reduced number of bobbins. The electrodes (1.5 m) of the pulse trawl targeting shrimp have a mutual distance of 0.6 m and generate 4.5 pulses a second of 0.5 ms each and a peak voltage of 60 V. The electrotrawls targeting sole have electrodes (9 m) on a mutual distance of 0.4 m with alternating isolated and conducting parts, generating 40–80 bipolar pulses a second of 0.25–0.38 ms each and a peak voltage of 45–50 V. A detailed description of the rigging of both electrotrawls, targeting shrimp or sole, and their pulse settings was reviewed in Soetaert *et al.* (2013). These electrical pulses generated by electrodes affect the target species more selectively than beam trawling, thus reducing both bycatch and fishing effort (Soetaert *et al.*, 2013). Removing the tickler chains or reducing the number of bobbins addresses the main problems with beam trawling, i.e. seabed disturbance, drag resistance, and fuel inefficiency (Van Marlen *et al.*, 2014), as well as the discard problem. The discard volume can be reduced by up to 76% in electrotrawls targeting brown shrimp, depending on the implementation and the number of bobbins used (Verschueren *et al.*, 2014). The effect on discards of pulse trawls targeting sole is less clear so far, which is probably related to the variation in design between different pulse gears, the rigging, and the fishing grounds. Van Marlen *et al.* (2014) found a 61.6 and 43.9% reduction in benthos discards and fish discards measured in weight per hour, respectively, whereas Rasenberg *et al.* (2013) in a more extensive comparison found no effect or a minor effect on plaice and sole discards, and a 16 and 42% reduction in the number of starfish and crabs caught, respectively.

In 1988, the use of electricity to catch marine organisms was prohibited by the European Commission (EC no. 850/98, article 31: non-conventional fishery techniques). But in 2009, Member States were granted a derogation by which 5% of the fleet was allowed to use pulse trawls in the southern part of the North Sea. Over 50 vessels have adopted this technique commercially, most of them with a Dutch licence. Although most vessels differ in rigging and weight of fishing gear, the electrical parameters are similar and can be roughly divided into two types of pulse. The majority, used to target flatfish, particularly Dover sole (*Solea solea* L.), uses a bipolar cramp pulse of 40–80 Hz to increase the catch efficiency. Only a few vessels target brown shrimp by outfitting their boat with electrotrawls that produce a unipolar startle pulse of 5 Hz. Before this fishery can be implemented, several concerns about negative effects of pulse fisheries on survival, behaviour, and reproduction of target and non-target species need to be addressed (ICES recommendations, 2009).

One of the concerns is the possible negative impact of the electrical pulses on invertebrates. Studies evaluating the effects of electrical pulses on invertebrates are limited and restricted to the pulse used to catch sole (i.e. 60–80 vs. 5 Hz for sole and shrimp, respectively). Smaal and Brummelhuis (2005) exposed 19 species of molluscs, echinoderms, crustaceans, and polychaetes to electrical pulses with an amplitude that was two times higher and an exposure time of eight times longer than the settings used in practice on commercial vessels targeting sole. Reactions during exposure were minor or negligible and the survival after 3 weeks did not deviate from the control group. van Marlen *et al.* (2009) exposed a selection of six benthic invertebrates to three subsequent bursts of 1 s at different distances from the electrode, ranging from 0.1 to 0.4 m. Compared with the control groups, they observed a significant reduction in the survival rate of exposed king ragworm (*Allita virens* S.) and European green crab (*Carcinus maenas* L.) of 3 and 5%, respectively, when all exposures were clustered. Atlantic razor clam (*Ensis directus* L.) displayed a significant 7% reduction in the survival rate near the electrodes but a better survival when exposed further than 0.2 m from the electrodes. The food intake was significantly reduced with 10–13% for the European green crab only. No significant effects were found for common prawn (*Palaemon serratus* L.), surf clam (*Spisula solidissima* L.), and common starfish (*Asterias rubens* L.). This made both abovementioned research groups conclude that, for the electrical pulses used to catch sole, it is plausible that the effects of pulse beam trawling are far less invasive than those of conventional beam trawling.

However, a full assessment of the possible side effects of electrical pulses should go beyond merely testing the sole pulse. Indeed, all parameters inherent to electrical pulses should be included in a more elaborate examination in which their values are varied and tested singly and in combination at various time points. Such information is indispensable to develop new types of pulses situated in a safe range for marine species and also to estimate the safety margin of the currently available commercial pulses (Soetaert *et al.*, 2013). Moreover, besides merely assessing mortality and aberrant behaviour, microscopic examination of the exposed invertebrates undoubtedly adds value when investigating the effects of electrical pulses. Indeed, sublethal effects with no immediate and direct impact may hereby be revealed. To our knowledge, no such studies have yet been performed. In this respect, the purpose of this study was to evaluate the effect of a broad range of electrical parameters and their combinations on invertebrates using behavioural analyses and data retrieval on mortality, complemented by macroscopic and microscopic observations.

Material and methods

Animals

In total, 1730 brown shrimp (*Crangon crangon* L.) were included in this study. The shrimp were caught 2 d before performing the experiments. The minimum length of the exoskeleton of all individuals in the study was 55 mm. For the first series of experiments, 650 animals were captured off the Belgian coast using the research vessel Simon Stevin, equipped with a 4-m shrimp beam trawl. Only short (± 20 min) fishing hauls were carried out to reduce stress and injury caused by the fishing process. The 1080 brown shrimp for the second series of experiments were caught using a man-towed beam trawl in the surf off the Ostend beach. After trawling, the catch was sorted and shrimp were immediately stored on a wet towel in covered containers with limited airflow. They were transported to the housing facilities within 3 h of catch.

Aquacultured ragworms ($n = 616$) were purchased from a commercial farm in the Netherlands (Topsy Baits, Wilhelminadorp, Netherlands) and acclimatized in the experimental facilities for 1 week before starting the experiments. The minimum length of the animals included was 8.5 cm.

Housing facilities

The animals were randomly divided into different experimental groups of 30–60 (shrimp) or 23–50 (ragworm) individuals and

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housed in a series of 18 PVC aquaria (0.75 m L × 0.55 m W × 0.30 m H) with a water level of 0.2 m. Each tank was provided with aeration and a cover with limited light penetration to mimic natural conditions. Natural seawater was used and the water quality was monitored daily. The following values were recorded: 15°C temperature; 35‰ salinity; 4.29 S m^{-1} conductivity; 8 pH; 6° KH total alkalinity; <25 mg l^{-1} nitrate; <0.2 mg l^{-1} nitrite; <0.1 mg l^{-1} ammonia. The bottom of the aquaria used for shrimp was covered with a layer of 10 mm rinsed sand (Ø1–2 mm), whereas no substrate was added for ragworm. The brown shrimp in each tank were fed with equal amounts of mussels and/or thawed ragworms three times per week. Any uneaten feed was removed 2 d after feeding. Ragworms received no feed.

Experimental design

Plate-shaped electrodes were used to minimize variability and ensure a standardized design. This type of electrode results in a homogenously distributed electrical field with a constant electrical field strength value between the electrodes. All pulses were generated by a laboratory pulse generator (LPG, EPLG bvba, Belgium) with a maximum output of 150 V, 280 A, and 42 kW peak. All the parameters were controlled by the included computer software. The generator was also equipped with a feedback system to ensure that the output exactly matched the set values. The output was double checked using an oscilloscope (Tektronix TDS 1001B).

The LPG was connected to the plate electrodes (0.55 × 0.4 × 0.001 m), placed at the short ends of the exposure tank, so on a mutual distance of 0.75, through two isolated cupper conductors (Ø = 20 mm^2). Stainless steel electrodes were applied to limit the distortion of current flow, to guarantee resistance to corrosion, and to minimize the release of metal ions (Stewart, 1972). Brown shrimp were exposed to nine pulse parameter combinations in two experiments (Table 1). Experiments adopting the first four combinations were performed in triplicate at three time points. The five other combinations were included in trials carried out in triplicate at a single time point. Ragworm was exposed to 11 parameter combinations. Each group contained 23–50 animals. For both model species, two control groups were included. Control group animals were treated exactly the same as the others, except that the electrical field was not activated.

Before the exposure of shrimp, the cover of the tank was removed and the electrodes were gently inserted in the water to minimize disturbance of the animals present in the tank. Ten seconds later, the animals were exposed to the electric pulse. After the shrimp resettled in the sediment, the electrodes were removed and the tank cover was replaced. For ragworms, the specimens were ladled out of the housing tank using a small net, exposed to the electric pulse in another tank, then moved back to the housing tank 30 s later. The behaviour of both species was monitored by a video camera (Sony ExmorR Handycam 12 Mp) during the 10 s after the electric pulses were applied. The reactions were scored based on the type and/or intensity of their reaction (Table 1). All animals were monitored until 14 d post exposure. Dead individuals and moults of shrimp were removed daily, and the number and the exoskeleton size of dead shrimp were measured and recorded. On day 14 post exposure, 10 randomly chosen animals from each group were sacrificed, measured, and processed for histological examination.

Pulse parameters

Five pulse parameters were used. The electrical field strength (E, V m^{-1}) indicates the voltage drop per unit of distance in the water. The frequency (F, Hz) signifies the number of pulses per second, while the pulse duration (D, μs) gives the duration of a single pulse in time. The pulse shape (S) describes the shape of a single wave, which may be square, exponential, or quartersinus as illustrated in Figure 1. The last parameter is the pulse type (P), which indicates the polarity of the pulses. Three pulse types were used: pulsed direct current (PDC) with monopole pulses, pulsed alternating current (PAC) with a positive and negative part in each pulse, and a bipolar pulse with alternating a positive and a negative pulse (PBC), as illustrated in Figure 1. Additionally, also the duty cycle (dc, %) is mentioned, which combines the frequency and pulse duration giving the time proportion during which electrical current runs between the electrodes or through the animal. Finally, two other parameters were also included: the exposure time (T, s), which is the total timespan during which electrical pulses were applied, and the number of exposures. The range in which these parameters were varied is given in Table 2.

The nominal pulse settings were 60 Hz, 150 V m^{-1}, 250 μs with a square pulse shape, which is similar to the sole pulse. In each experiment, only one of the parameters was changed to enable the linking of possible effects to that parameter. The exposure time was 2 s, except during the periodical exposures (P), where animals were exposed during 1 s repeatedly on days 0, 5, 8, and 11 to simulate possible repetitive exposures of animals in the field. As indicated in Table 2, ragworm was exposed four times during the first 10 d to a pulse in which all the parameters were set to the maximum (R), whereas brown shrimp was exposed repeatedly to the pulses used in the shrimp fishery (R_{cr}) and the flatfish fishery (R_{fl}).

Table 1. Scoring of reaction of brown shrimp and king ragworm during and up to 10 s after exposure to electric pulses.

Exposure	Score	Brown shrimp	Ragworm
During	0	No reaction	No reaction
	1	<10% jumps	Minority squirms
	2	10–50% jumps	Majority squirms
	3	50–90% jumps	100% Squirms, minority strongly
	4	>90% jumps	100% Squirms, majority strongly
	5	100% jumps	100% Strongly squirms
After	0	No reaction	No reaction
	1	All burrowed immediately	Minority squirms
	2	All burrowed after 1 s	Majority squirms
	3	All burrowed after 2 s	100% Squirms, minority strongly
	4	All burrowed after 3 s	100% Squirms, majority strongly
	5	Some still jumping >3 s	100% Strongly squirms

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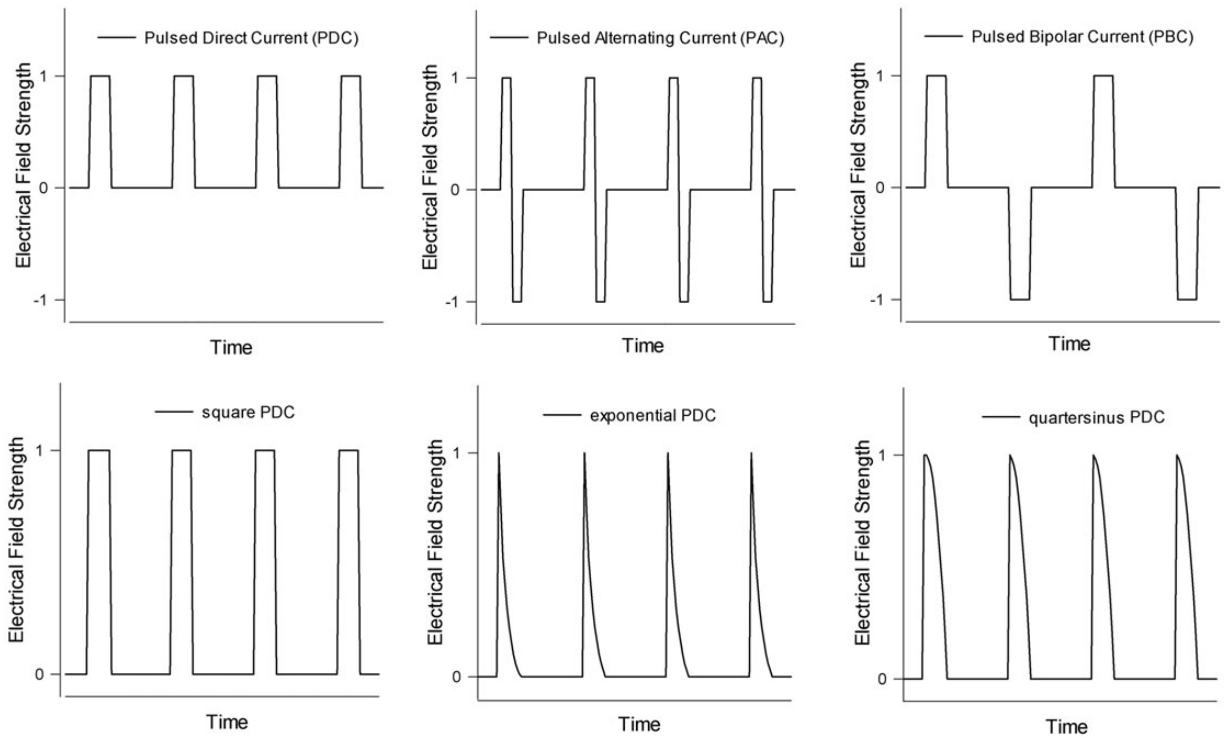

Figure 1. An illustration of the three pulse types and three pulse shapes used during the experiments.

Table 2. Overview of all the tested electrical pulses with their respective settings for each parameter in shrimp (sh) and ragworm (ra).

Pulse ID	*E* (V m^{-1})	*F* (Hz)	*D* (μs)	dc (%)	*P*	*S*	*T* (s)	*n*	Species
Ctr	0	0	0	0	–	–	2	1	sh + ra
F5	150	5	250	0.1	PDC	s	2	1	sh + ra
F60	150	60	250	1.5	PDC	s	2	1	sh + ra
F200	150	200	250	5.0	PDC	s	2	1	sh + ra
E60	200	60	250	1.5	PDC	s	2	1	sh + ra
D60	150	60	1000	6.0	PDC	s	2	1	sh + ra
PAC60	150	60	250	1.5	PAC	s	2	1	sh + ra
PBC60	150	60	250	1.5	PBC	s	2	1	sh + ra
S_e60	150	60	250	1.5	PDC	e	2	1	ra
S_q60	150	60	250	1.5	PDC	q	2	1	ra
T60	150	60	250	1.5	PDC	s	5	1	ra
R	200	60	1000	6.0	PBC	s	2	4	ra
R_{cr}	150	5	500	0.3	PDC	s	1	4	sh
R_{fl}	150	80	220	1.8	PBC	s	1	4	sh

E, electrical field strength; *F*, frequency; *D*, pulse duration; dc, duty cycle; *P*, pulse type; *S*, pulse shape; *T*, exposure time, *n*, number of exposures; PDC, pulsed direct current; PAC, pulsed alternating current; PBC, pulsed bipolar current; s, square shaped pulse; e, exponentially shaped pulse; q, quarter sinus shaped pulse; sh, shrimp; ra, ragworm.

Gross and histological examination

Two weeks following exposure to the electrical pulses, all animals were sacrificed by injection of formaldehyde (brown shrimp) or an overdose of clove oil in the water (ragworms). The animals were examined for gross lesions and their length was measured. The brown shrimp were processed according to the protocols of Bell and Lightner (1988) and Hopwood (1996). Briefly, the carapax and tail of 10 individuals per group were separated and fixed in Davidson fixative (Bell and Lightner, 1988; Hopwood, 1996) for routine paraffin embedding and sectioning. Tissues were dehydrated in graded alcohol and embedded in paraffin wax. Transversal sections of 5 μm thickness were cut with the microtome using the section transfer system (Microm, Prosan, Merelbeke, Belgium). The sections were stained with haematoxylin/eosin and examined with a special focus on the epithelium of the cardiac stomach, the hepatopancreas, and the cardiac and caudal muscles. In addition, the severity of an intranuclear bacilliform virus (IBV) infection in the hepatopancreas, described in brown shrimp by Stentiford *et al.* (2004), was scored blind in five stages based on its severity (0–4): 0 = absent; 1 = scattered (few aberrant nuclei and most hepatopancreatic tubuli not affected); 2 = frequent (frequent aberrant nuclei present in many hepatopancreatic tubuli); 3 = abundant (most hepatopancreatic tubuli contain few aberrant nuclei); 4 = severe (most hepatopancreatic tubuli contain many aberrant nuclei).

Ten ragworms per group were fixed in 4% formaldehyde in seawater solution, processed, and stained as described for shrimp. Three sections per animal were examined to visualize different body zones: pharynx (3rd + 4th segments), oesophagus (7th + 8th segments), and intestine (11th + 12th segments) regions. Special attention was paid to the ganglion, the body wall, the gut, and the parapodial, circular, and lateral muscles. The number of animals in which melanomacrophage aggregates (MMAs) were encountered was recorded. In addition, the number of MMAs in each of the aforementioned organs was counted blind per section and scored from 0 to 3 as follows: 0 (no MMA), 1 (1–2 MMAs), 2 (3–5 MMAs), or 3 (>5 MMAs).

Statistical analysis

For brown shrimp, the percentage mortality rate after 7 and 14 d, the average size difference between dead and surviving individuals, and

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the mean virus load of IBV were obtained per aquarium and analysed using a mixed model with pulse exposure as a categorical fixed-effects factor and replicate as a random effect. Tukey adjusted p-values were used for the evaluation of all pairwise comparisons.

For ragworm, the analysis of the observed mortality after 14 d was based on the exact logistic regression model. The number of animals affected with MMAs in each group was analysed based on Poisson's regression model and the average values given for the scored presence of MMA were analysed based on the logistic regression model.

Results

Brown shrimp

Reaction to the exposure

The results are summarized in Table 3. In general, 95–100% of the shrimp reacted with a tail flip when exposed to electrical pulses. The reaction was correlated with the frequency: shrimp exposed to 5 Hz showed tail flipping on every pulse and jumped in random directions, often reaching the surface of the water (0.2 m). A frequency of 60 or 200 Hz and higher resulted in one powerful contraction that made them jump 0.1–0.15 m out of the sediment. This cramp persisted during the entire exposure and resulted in the shrimp turning upside-down and sinking on their back to the sediment after 1–1.5 s. Within 0.25 s after the exposure, all shrimp showed tail flip escape behaviour, which lasted longer in shrimp that had been exposed to a more intense electric field. None of the control animals displayed a tail flipping reaction during the observation and all animals remained buried in the sand.

Effects of exposure

During the first days, all shrimp displayed a distinct fright reaction when the cover of the tanks was removed. All animals also actively searched for food after it was dropped into the water and ate all the feed provided. However, after 7–10 d, a decrease in activity was noted and more food was left uneaten, both for the exposed and for the control animals. At the same time, an increase in the mortality rate was observed in all groups (Table 3). None of the tested parameter combinations resulted in a significantly higher 7-d or 14-d mortality. In addition, no difference in size was observed between animals that died during the experiment and those that survived.

No external lesions were observed in any of the shrimp nor were any lesions observed upon histological examination. Significantly higher scores for IBV inclusions were found for the group exposed to the highest field strength of 200 V m^{-1} (E60) when compared with the control group ($p_{adj} = 0.0166$), the PAC60 group ($p_{adj} = 0.0255$), and the F200 group ($p_{adj} = 0.0049$). When compared with Db60, the difference was not significant ($p_{adj} = 0.1523$). No significant increase compared with the control group was found in the second experiment. However, the disease prevalence of shrimp caught at sea for the first experiment was lower (52% infected, average score = 0.64 ± 0.86) than shrimp caught by foot trawl at the coastline for the second experiment (67% infected, average score = 1.07 ± 1.00). This may be due to environmental stressors such as pollution (Stentiford and Feist, 2005), with pollutants generally reaching concentrations in coastal water. This effect might have been strengthened by Ostend's harbour mouth, which was located <500 m from the beach where the shrimp were foot trawled.

Ragworm

Reaction to exposure

During and immediately after exposure, squirming was the only reaction observed in ragworm. Regardless of the frequency used, no cramp reactions were seen. The intensity of squirming during exposure was variable, with no apparent correlation with the pulse parameters. Although variable, the post-exposure behaviour increased with duty cycle. Animals exposed to duty cycles lower than 1.5% exhibited minor squirming, whereas ragworms exposed to duty cycles of 5% and higher and to the highest field strength showed intense squirming. The control animals sometimes showed minor squirming as a response to the mechanical stimulation of their displacement, but no aberrant behaviour was seen.

Effects of exposure

The results are summarized in Table 4. During the 14-d follow-up period, the mortality varied between 0 and 4%. None of the tested parameter combinations resulted in significantly greater mortality than the control group. No gross lesions were observed nor were any abnormalities detected histologically. No significant differences were noted in the number of animals per group in which MMA were observed nor in the mean MMA score of animals in between groups.

Table 3. Results of behaviour scoring during and after exposure, the survival rate at 7 and 14 d after exposure, and the severity scoring for intranuclear baciliform viruses (IBV) after exposure of brown shrimp to different electric pulses.

		Median behaviour score (± s.d.)		Mean percentage survival (± s.d.)		
Study[a]	**Pulse ID**	**During**	**after**	**7 d**	**14 d**	**Mean IBV score (± s.d.)**
1	Ctr	0 (±0)	0 (±0)	82.6 (±9.8)	65.0 (±20.6)	0.5 (±0.9)
	F200	5 (±0)	4 (±1)	85.3 (±9.1)	62.5 (±23.0)	0.3 (±0.6)
	E60	5 (±1)	4 (±1)	83.0 (±12.6)	63.6 (±21.2)	1.4 (±1.2)
	D60	5 (±0)	4 (±0)	77.8 (±14.0)	57.1 (±22.7)	0.7 (±0.7)
	PAC60	5 (±1)	4 (±0)	82.0 (±7.1)	55.2 (±18.1)	0.6 (±0.7)
2	Ctr	0 (±0)	0 (±0)	91.7 (±2.4)	70.0 (±2.4)	1.2 (±1.1)
	F5	5 (±1)	2 (±0)	92.8 (±2.8)	77.2 (±6.1)	0.7 (±0.7)
	F60	5 (±1)	4 (±1)	92.2 (±3.4)	68.9 (±4.4)	0.8 (±1.0)
	PBC60	5 (±1)	3 (±1)	92.8 (±1.6)	72.8 (±6.9)	1.2 (±0.8)
	R_{cr}	5 (±0)	3 (±1)	93.3 (±4.1)	66.1 (±9.5)	1.5 (±1.0)
	R_{fl}	5 (±0)	3 (±1)	95.6 (±0.8)	75.6 (±7.5)	1.0 (±1.1)

[a]The first study was repeated at three time points; the triplicates of the second study were performed simultaneously.
s.d., standard deviation.

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Table 4. Results of survival, presence, and scoring of MMAs 14 d after exposure of ragworm to different electric pulses.

Pulse ID	# of animals	14-d survival (%)	MMA Presence (% animals)	MMA Mean score (±s.d.)
Ctr	50	96.0	80	1.1 (±0.74)
Ctr*	50	100.0	70	1.2 (±0.82)
F5	30	100.0	40	0.9 (±1.23)
F60	50	98.0	40	0.4 (±0.69)
F200	30	100.0	60	1.2 (±1.49)
D60	29	100.0	60	0.7 (±0.63)
E60	30	100.0	70	1.2 (±0.99)
PAC60	50	98.0	50	0.5 (±0.50)
PBC60	50	98.0	60	0.7 (±0.67)
S_e60	23	100.0	10	0.4 (±1.26)
S_q60	23	96.7	60	1.1 (±1.25)
T60	30	100.0	60	0.7 (±0.67)
Chr	50	98.0	60	1.0 (±0.88)
Chr*	50	100.0	50	0.7 (±0.82)

Experiments indicated with "*" were done with ragworms that were not fed during 1 month before exposure.

Discussion

Experimental set-up

For several reasons, brown shrimp and ragworm were chosen as model species for the taxa crustaceans and polychaetes, respectively. First, both benthic taxa live in close association with the seabed and are therefore very likely to be exposed to electrical pulses during electrotrawling. Second, van Marlen *et al.* (2009) demonstrated that these taxa appeared to be the most sensitive to electric pulses. Third, both species are an important food source for various fish species, in particular flatfish, which are targeted in commercial fisheries (Beyst *et al.*, 1999; Schuckel *et al.*, 2012). These species therefore have an indirect economic value and play an important role in the foodweb. In addition, brown shrimp also have a direct commercial importance: the total annual landings of this species exceed 30 000 tons in the North Sea (ICES, 2013). A practical consideration was that both species can easily be obtained in large numbers, which was a necessary prerequisite to conduct these elaborate experiments.

To allow for a standardized design with minimal variability, plate-shaped electrodes were used. The use of such electrodes results in a homogenous distribution of the electrical field with constant electrical field strengths between the electrodes. This is in contrast to the cylindrical, wire-shaped electrodes used in the field. This study strived for worst-case exposures to avoid underestimation of elicited effects, hence allowing to set the boundaries for a safe range of pulse parameters that can be employed in electrotrawling. Rather large model species and large-size individuals within these species were chosen to maximize the difference in electrical potential experienced by the animal. Furthermore, the electrical pulses in the experiments were increased to levels above those used in electrotrawls, except for the field strength. The nominal field strength in our experiments was 150 V m^{-1} with a tested maximum of 200 V m^{-1}. In the field, field strengths may exceed 200 V m^{-1} at a distance closer than 0.04 m from the electrodes. This implies that part of the vertically buried ragworm or a jumping shrimp may be exposed to higher field strengths during a very short time span. However, in our study, the entire body was exposed during 2 s and frequencies and pulse durations were at least doubled compared with the commercial flatfish pulse trawlers, resulting in duty cycles up to 6%, whereas the maximum duty cycle in the field is 1.76% (Soetaert *et al.*, 2013). This signifies that the electrical pulses to which the invertebrates were exposed had an energy content of up to four times higher than the pulses currently used in the field.

Reaction of the invertebrates

Exposure to 5 Hz pulses induced a tail flip response in brown shrimp, as described by Polet *et al.* (2005a), with abdominal muscles contracting following the 5-Hz rhythm. This finding is consistent with adopting this same frequency to stimulate buried shrimp and make them jump out of the sediment in commercial electrotrawling for brown shrimp (Polet *et al.*, 2005a, b). However, when the frequency was increased to 60 Hz and higher, only one persistent contraction was observed. This phenomenon is also present in vertebrate species and may be explained as an overstimulation of the muscle. At 60 Hz and higher, the contractions of the muscle induced by the electrical pulses occur in very quick succession, so that the muscles remain contracted. This leads to cramping of the muscle and subsequent immobility. The threshold for this cramp reaction in vertebrate species is ∼20 Hz (Snyder, 2003). Shrimp showed immediate (1–3 s after exposure) recovery as shown by clearly observed escape behaviour, immediately followed by burrowing in the sediment. The single strong upwards jump seen at higher frequencies followed by escape behaviour might offer an alternative way to catch brown shrimp using electrical pulses. The current electrofishing on sole is based on pulses with frequencies of 40–80 Hz. A combi-pulse may stimulate both shrimp and sole and enable their catch with the same fishing gear. However, the jump height was limited and may be lower in the field, because the animals may be buried deeper in the sediment. Additionally, the escape behaviour has a very short duration.

The observed cramp reaction followed by immediate recovery seems to be common for crustacean species. Indeed, European green crab, common hermit crab (*Pagurus bernhardus* L.), and helmet crab (*Corystes cassivelaunus* L.) also showed a cramp reaction followed by direct recovery when exposed to the cramp pulse used in the flatfish fishery (Smaal and Brummelhuis, 2005; van Marlen *et al.*, 2009). Only common prawns stayed immobilized until 1 min after exposure (Smaal and Brummelhuis, 2005; van Marlen *et al.*, 2009). This cramp reaction is not observed in other invertebrates. Echinoderms (starfish, sea urchin, and brittlestar) show no reaction, whereas razor clams can even use their foot and siphon, often exhibiting strong enough reactions to even propel them away from the pulse (Smaal and Brummelhuis, 2005; van Marlen *et al.*, 2009). Molluscs (cockle, prickly cockle, whelk, netted dog whelk, and subtruncate surf clam) retreat into their shell and close it during exposure. This may also be assigned as a cramp reaction, but they display immediate recovery (Smaal and Brummelhuis, 2005). Both studies used the cramp pulse of the sole pulse gear, which is very similar to PAC60 and PBC60 and R_{cr}, but unfortunately neither study specified the pulse parameters used.

Previous studies report a variable reaction of annelids exposed to electric pulses. Smaal and Brummelhuis (2005) observed no reaction in ragworm and sea mouse (*Aphrodita aculeate* L.). This is in contrast to van Marlen *et al.* (2009) who observed a clear reaction in 50% of the ragworm exposed at a distance closer than 0.2 m from the electrodes. The post-exposure reaction of ragworm in our experiments was generally more intense when exposed to either a high amplitude pulse or duty cycles of 5% and higher. However, ragworm showed hardly any reaction to electric pulses with duty cycles of 1.5% and lower, which is the range in which

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sole pulse trawlers operate in the field and in which previous studies have been done. Moreover, because ragworms are burrowed in the sediment, they would automatically experience lower field strengths due to their distance and orientation, which again stresses the worst-case character of our experiments. This reasoning also applies to brown shrimp. Moreover, the brown shrimp in these experiments were exposed in the group, which results in higher field strengths inside the body compared with the exposure of a single animal, as described by D'Agaro and Stravisi (2009) with seawater fish.

Effects of exposure

To our knowledge, this is the first study including histological examination evaluating the effects of electric pulses on animals. This technique makes it possible to disclose sublethal effects that are not macroscopically discernible. No lesions were observed in any of the examined samples. This may indicate that microscopic injuries caused by short exposures to electrical pulses were either absent or had healed 14 d following exposure for brown shrimp and also for ragworm. However, scoring the severity of IBV infection revealed an increase in brown shrimp exposed to the highest field strength (200 V m^{-1}), although no pathological manifestation of the IBV infection was yet evident. Increasing levels of severity of IBV infection may be caused by environmental stressors (Stentiford and Feist, 2005). This is further reinforced by studies of vertebrate species, in which stress has been stated as the most important factor for latent infections to eventually manifest as a disease (Sindermann, 1979). The finding in the current study might suggest that a 2-s exposure to field strengths of 200 V m^{-1} or higher can be regarded as a type of stressor. No significant increase was noted when the other pulse parameters were increased at a lower field strength. Note that, in the field, such high field strengths are only found in a very narrow zone around the quickly moving electrodes, which means that only a small minority of shrimp will be exposed to a similar pulse; furthermore, the duration of that exposure would also be shorter. Despite the lower and shorter exposure to electric pulses in the field, this result undoubtedly warrants further research to better understand the mode of action and to explore a possible dose–response effect.

During the 2-week monitoring period in the laboratory, none of the exposed brown shrimp and ragworm showed increased 14-d mortality or gross lesions, even when exposed to pulses up to four times in 10 d. Testing repeated exposure is important because the periodic exposure of these animals on popular fishing grounds must not jeopardize the stocks of invertebrates, both for ecological and for (in)direct commercial reasons. When comparing our outcomes with previous exposure experiments on invertebrates, our results confirm those obtained by Smaal and Brummelhuis (2005) with 19 invertebrate species, although only three of these species had more than 15 individuals exposed. In contrast, van Marlen *et al.* (2009) did find minor effects on survival. When exposed far from the electrodes, ragworm and green crab exhibited 5% lower survival, whereas razor clam showed 6% higher survival. When exposed at 0.2–0.3 m from the electrodes, European green crab and razor clam displayed 7.5% lower and 6% higher survival, respectively. Nearby the electrodes (0.1–0.2 m), no increased mortality was found for European green crab, whereas razor clam and ragworm had a 7% lower survival. When all groups were clustered, a 3–5% lower survival was found for ragworm and European green crab, respectively, when exposed to the flatfish pulse. No correlation between higher electrical field strengths and an increase in mortality was found. No significant effect was found on common starfish, common prawn, and surf clam. However, the significant increase in survival of razor clam suggests that unsufficient animals were included to exclude the variability due to high natural mortality, so these results should be interpreted with caution. In our study, which included more animals per group and higher electric loads were adopted, no negative impact on survival was demonstrated. Our data thus indicate that this alternative fishing technique is worthy of further research, both basic and applied. These short-term experiments can be viewed as a prelude to further research on the long-term effects of electric pulses on growth, reproduction, and behaviour. The latter would require keeping and breeding the animals in captivity, which is by no means straightforward in brown shrimp, as evidenced in the current and previous studies (Verhaegen, 2012). In the 10 d following the onset of the experiment, mortality gradually increased in all groups regardless of the treatment. For that reason, mortality rates were compared on day 7 and day 14 post-exposure.

Conclusion

Brown shrimp and king ragworm were used as model species to investigate the short-term effect of electrical field strength, pulse frequency, pulse duration, pulse type, exposure time, and pulse shape (only ragworm) on benthic invertebrates. Although a broad range of different parameters was examined, none of these resulted in gross lesions, histological changes, or increased mortality at 14 d after exposure, rectifying the promising character of this alternative fishing technique. However, an increase in severity of IBV infection was found in brown shrimp exposed to the highest electrical field strengths, warranting further research.

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