UNIVERSITEIT GENT

FACULTY OF **VETERINARY MEDICINE**
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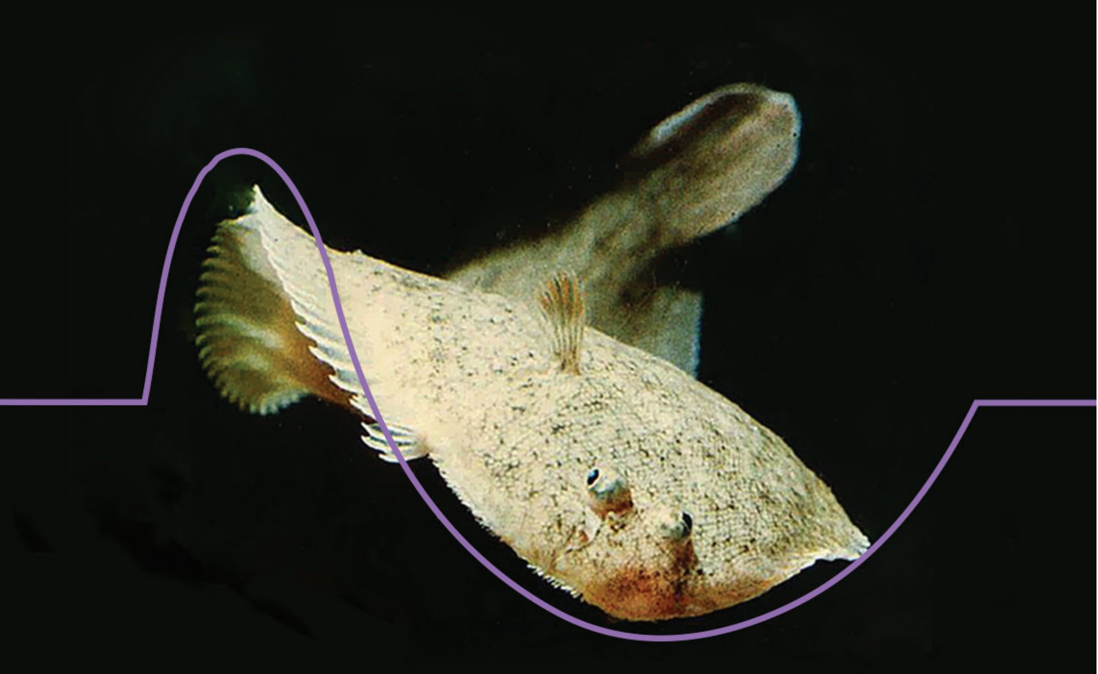
# ELECTROFISHING:
# EXPLORING THE SAFETY RANGE OF ELECTRIC PULSES FOR MARINE SPECIES AND ITS POTENTIAL FOR FURTHER INNOVATION



**MAARTEN SOETAERT**

ELECTROFISHING: EXPLORING THE SAFETY RANGE OF ELECTRIC PULSES
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agency for Innovation
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ILVO
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Electrofishing: exploring the safety range of electric pulses for marine species and its potential for further innovation.

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ir. Maarten Soetaert

# ELECTROFISHING: EXPLORING THE SAFETY RANGE OF ELECTRICAL PULSES FOR MARINE SPECIES AND ITS POTENTIAL FOR FURTHER INNOVATION

# ELEKTRISCH VISSEN: HET VERKENNEN VAN DE VEILIGE GRENZEN VAN ELEKTRISCHEPULSEN VOOR MARIENE DIEREN EN ZIJN POTENTIEEL VOOR VERDERE INNOVATIE

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*We don't see things as they are, we see them as we are.*

Babylonian Talmud

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# ABBREVIATION INDEX

| | |
|---|---|
| A | Amplitude (in Volts [V] or Volts per meter [$V\ m^{-1}$])<br>maximum/minimum potential difference on the electrodes. |
| AC | Alternating Current<br>type of electric current in which the direction of the flow of electrons switches back and forth at regular intervals or cycles. |
| BRP | Benthos Release Panel |
| dc | Duty Cycle (in percentage, [%])<br>percentage of the time during which current is running: dc = F x D |
| DC | Direct Current<br>type of electric current which flows consistently in one direction |
| DPFE | Days Post First Exposure |
| D | pulse Duration (in microseconds, [µs])<br>the duration of a single pulse |
| E | Electric field strength (in Volts per meter, [$V\ m^{-1}$])<br>the voltage drop per unit of distance in the water |
| F | Frequency (in Hertz, [Hz])<br>the number of pulses per second |
| FEMM | Finite Element Method Magnetics<br>a software packet solving 2D problems in magnetics and electrostatics |
| I | electric current (in Ampère, [A])<br>the amount of electrons (in coulomb) that moves per second |
| ICES | International Council for the Exploration of the Sea |
| ILVO | Belgian Institute for Agricultural and Fisheries Research |
| L | exposure Length or time (in seconds, [s])<br>the time period during which the animal was exposed |

| | |
|---|---|
| MLS | Minimum Landing Size<br>the species specific minimum length to be landed and sold |
| MMA | MelanoMacrophage Aggregates<br>aggregates of macrophage-like pigmented cells |
| P | electric Power (in Watt, [W])<br>the rate at which electric energy is transferred: P = U x I |
| PAC | Pulsed Alternating Current<br>pulses in which the direction of electrons switches one time resulting in a pulse shape with a positive and negative part in each pulse |
| PBC | Pulsed Bipolar Current<br>pulses which alternatingly switch from direction of flow resulting in alternating positive and negative pulse shapes separated in time |
| PDC | Pulsed Direct Current |
| PE | PolyEthylene |
| S | pulse Shape<br>the shape of a single pulse, e.g. exponential, square, quarter-sinus… |
| T | pulse Type<br>indicates the polarity of the electric pulses, e.g. PAC, PBC or PDC |
| TAC | Total Allowable Catch<br>maximum quantity of a certain fish species that fishermen are allowed to catch and land |
| U | potential difference (in Volts, [V])<br>the difference in potention on two elctrodes or the amplitude of the pulse |
| VMS | Vessel Monitoring System<br>This software tracks the speed and location of commercial ships. |

# PREFACE

The term ‘electrofishing’ has been used since the 1950’s to appoint a frequently used sampling technique for fish in freshwater whereby electric energy is passed into the water. In case direct current (DC) is used, fish intercepting this energy will show forced swimming toward the source of electricity, which is called galvano-taxis. This is believed to be a result of direct stimulation of the central and autonomic nervous system which control the fish’s voluntary and involuntary reactions. When approaching the anode, fish will in succession show quivering or pseudo-forced swimming, narcosis and tetany. The unconscious fish will rise to the water surface, enabling an easy catch and handling (for species determination, weighing and measuring of the fish). In case alternating current (AC) is used, fish will not show galvano-taxis, but show immediate immobilization through narcosis and tetanus which has the disadvantage that the fish may be at unreachable depth or distance from the boat. Both types of current may be periodically interrupted (pulsed) in water to cope with increasing power demands (Snyder, 2003a).

As reviewed by Snyder (2003a), freshwater electrofishing is a very effective sampling method but it has the disadvantage that it may inflict harm to fish. Salmoninae are known to be susceptible to spinal injuries, associated haemorrhages, whereas it can be lethal for burbot and sculpins under some conditions. Freshwater electrofishing is also reported to result in cardiac arrests, long behavioural and physiological recovery times and doubtful effects on early life stages. Unfortunately, many questions remain unanswered, the interpretation of some results is often difficult to understand or questionable and a lot of variation and contradictions are reported. This is not surprising since application of electric pulses comprises many different factors: electrode shape and set-up, different pulse parameters used, differences in conductivity, temperature and surrounding medium, size of the animal, species-dependent reactions and side-effects,… Moreover, electrofishing involves a very dynamic and complex mix of physics, physiology and behaviour which remain poorly understood, despite the considerable amount of research that has been performed.

In recent years, electricity is also used in seawater to increase the catch efficiency for certain species. For these applications, fish are exposed for less than 2 seconds to multiple short pulses. Depending on the number of pulses used per second (frequency [Hz]), animals will show different (behavioural) reactions ranging from a startle or escape response at frequencies below 20 pulses per second (20 Hz) to a cramp reaction when more pulses per second/higher frequencies are used. Based on these findings, different pulses are designed allowing shrimp to jump up from the seafloor (shrimp startle pulse) or to immobilize sole by inducing a muscular cramp response. As a result, the catch rate of these animals in the net, which is trawled immediately behind the electrodes, is improved. These electrotrawls differ from freshwater electrofishing in aimed reaction, working principle, pulse settings and gear, as overviewed in Table I. Note that this table does not include marine electrofishing on *Ensis* spp. because it is poorly documented and the pulse settings (continues current, not pulsed) are more similar to freshwater electrofishing because it aims for a similar slow behavioural response in *Ensis* spp. and subsequently requires exposure times around 1 minutes.

How electric current interferes with the fish physiology is not yet elucidated. Fish can be considered to be an electrical network composed of resistors and capacitors. The membrane and tissues act as the dielectric of a capacitor with the ability to by-pass frequencies as well as frequency attributes expressed in the leading and trailing edges of the pulse (Sternin *et al.*, 1976; Sharber *et al.*, 1999). Given the differences in the anatomy of fish species, the response to an electric stimulus will differ across species (Halsband, 1967; Emery, 1984). The interaction with the electric field is also affected by the pulse settings and the environment. In addition, other pulse parameters can affect the impedance of tissues (Finlay *et al.*, 1978), resulting in different electric doses and effects. The conductivity of the surrounding medium is also decisive. Whereas in fresh water high amounts of current may flow through the fish' body as it conduct current better than the surrounding water, this will not occur in fish surrounded by seawater with a much higher conductivity (Lines and Kestin, 2004). On the other hand, much higher field strengths will be found in the immediate surrounding of a fish in seawater, which might indirectly affect the flow of ions in the fish' body, the charge on neurons, the polarity of membranes and tissues,…. The long list of differences and poorly understood phenomena stress that prudence is warranted when extrapolating freshwater results.

Table I: Overview of major differences between freshwater and marine electrofishing.

| | Freshwater electrofishing | Marine pulse fishing |
|---|---|---|
| Application | sampling of river or lakes | commercial trawling |
| Goal | sampling all fish species of all size | increase marketable catch |
| Working principle | inducing galvano-taxis to anode<br>or immobilization on the seafloor | upwards startle reaction<br>or immobilization on the seafloor |
| Gear | static | dynamic/moving |
| Electrodes | 2 (hemi)sphere, ring or cylinder | multiple wire-shaped electrodes |
| Electrode distance | > 1 m | 0,3 - 0,6 m |
| Water conductivity | 0,01-0,1 S $m^{-1}$ | 4,2 S $m^{-1}$ (North Sea, 15°C) |
| Electric dispersion | current = or > in fish than in water | current < in water than in fish |
| Exposure duration | 0,5-3 minutes | 0,5-3 seconds |
| Duty cycle | always >10%, often 60-100% | <3% |
| Frequency | 15-120 Hz (and up to 500 Hz) | 5-80 Hz |
| Potential difference | 100-400 V | 60-100 V |
| Pulse type | DC, PDC or PAC | always pulsed |
| Pulse shape | exponential, sinus, quartersinus, square, triangular,… | rounded shape caused by impedance of long electrodes |

Although electric stimulation can cause injuries and can be lethal, when an appropiate field intensity and duration of exposures are applied (Snyder, 2003a), a correct use of electric stimuli offers incredible opportunities and allows us to achieve catch results that outperform all other techniques, both for sampling in freshwater as for commercial fishing in seawater. This stresses the importance of studying the pulse settings and optimizing them in such a way that minimal harm and maximal performance can be balanced. The latter is especially important for marine species and marine electrofishing, as the available knowledge is very limited and extrapolation from freshwater results is difficult as consequence of the differences listed in Table I. Extensive research of side-effects of marine electrofishing was also premised by ICES before electrotrawls can widely be introduced (ICES, 2009). Therefore, this PhD thesis aspires to study commercially used electric pulses and to delimit their safe range of application for marine demersal species, within which no or acceptable side-effects are observed. Besides, it also evaluates the potential for a new application in which electric pulse stimulation is used for a further increased selectivity in fishing gears.

# CHAPTER 1

# MARINE ELECTRIC FISHING: A PROMISING ALTERNATIVE FISHING TECHNIQUE WARRANTING FURTHER EXPLORATION

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Adapted from:

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

# Introduction on trawl fisheries

Preoccupations on the potential negative effects of trawling on the seabed have existed almost as long as the fishing method itself, with early concerns being voiced by fishermen themselves dating back to the 14th century (Hovart, 1985). These concerns are increasingly gaining international public and political attention (Linnane, 2000). Especially the beam trawl fishery with tickler chains targeting dover sole (*Solea solea* L.) and plaice (*Pleuronectes platessa* L.) is an object of discussion due to its seafloor disturbance and the by-catch of benthic organisms (Lindeboom *et al.,* 1998; Bergman & van Santbrink, 2000; Jennings *et al.,* 2001; European Commission, 2011). Apart from direct physical disruption such as scraping, ploughing or re-suspension of the sediment (Jones, 1992; Fonteyne *et al.,* 1998), the bottom disturbance renders disturbed and damaged invertebrates susceptible to predation while colonies rooted in the sand are dislodged (Rabaut *et al.*, 2007).

Beam trawling is typically a mixed fishery, targeting different species at once and therefore often characterized by poor selectivity. This results in large amounts of by-catch, which is mainly discarded because it comprises undersized fish or non-marketable species. In its new policy for 2013, the European Commission has selected beam trawling as one of the first fisheries to implement the discard ban and for which unwanted by-catch should be reduced (Council of the European Union, 2011). The fact that e.g. shrimp beam trawling is carried out in vulnerable areas like coastal zones and estuaries, often important nurseries for a wide range of marine species, intensify the problem. Discarding of young fish can have a significant influence on the commercial fish stocks because the limited survival rates result in a loss of potential growth and contribution to stock replacement (Van Beek *et al.,* 1990; Revill *et al.*, 1999). The direct loss of potential income through the discarding of commercial species in the North Sea has been calculated for the Dutch beam trawl and UK roundfish fishery at 70% and 42% of the total value of the annual landings, respectively (Cappell, 2001). Revill *et al.* (1999) estimated that the annual lost landings arising from discarding in the North Sea brown shrimp fisheries only, had an estimated market value of over € 25 Million. This indicates very well the long-term economic potential of reducing discards. A 3d drawback of the traditional beam trawling is its fuel consumption: 2.5 to 4 L of gasoil is consumed for each kg fish that is caught (Heijer & Keus, 2001; Thrane, 2004; Polet *et al.*, 2010), resulting in 25-50% of the landing value needed for covering its fuel costs. Paschen *et al.* (2000) calculated that 65% of the gasoil is used to drag the gear over the seafloor and through the water and that 30% of the total towing resistance is caused by the tickler chains.

A promising alternative are electrotrawls, in which the mechanical stimulation by tickler chains or bobbins is replaced by electric stimulation with electrodes, inducing electric pulses. The removal of the tickler chains or reduction of bobbins results in reduced bottom contact, discards and fuel costs. Despite the fact that this application has been under investigation since the 1960's, the huge technical challenge, the limited knowledge in this field and legal constraints put off the commercial breakthrough of electrotrawls until 2009. Still, many questions about impact and possible side-effects remain unanswered. In this introduction, the rise of the marine electric fishing in the North Sea is discussed with emphasis on the recently developed commercial systems, the effects of electric pulses on marine species and the environment, and the opportunities and challenges of this alternative fishing technique for the future.

# Early developments in marine electrotrawls

Electricity has been used a first time by humans to kill, anesthetize, capture, drive, draw, tickle, guide, block or repel fish in the 1800's in freshwater (Vibert, 1967, Halsband and Halsband, 1984; Hartley, 1967). Already in 1863, a British patent was granted to Isham Baggs for electrofishing, but the widespread development and use of electrofishing did not occur until the 1950's (Hartley, 1967; Reynolds, 1995), when it became an important capture technique for population and community surveys in freshwater systems. Even today it is still a common technique due to its high sampling efficiency (Growns *et al.*, 2008). The first record of the use of electricity for seawater applications dates back to 1765 when the Dutchman Job Baster wrote "Would the electricity, which shocks are so similar to those produced by the electric eel, have no effects on shrimp? To my opinion, it's worth to investigate that" (de Groot & Boonstra, 1974). However, it took till 1949 before the interest in marine electrofishing was really stimulated by the successful introduction of electrofishing techniques in freshwater and experiments done in Germany, as reported by Houston (1949). In the subsequent years, the response of tropical marine fish (*Kuhlia sandvicensis* L.) (Morgan, 1951; Tester, 1952), sardines (*Sardinops sagax* J.) (Groody, 1952) and the pink grooved shrimp (*Penaeus duorarum* B.) (Highman, 1956) to electric pulses was investigated, but also the potential application of electrified hooks for tuna, electrified harpoons for whales, electric fences with fish magnets and spherical anodes with lights and fish pumps were subjects of research (Sternin *et al.*, 1972).. At that time, the aim was to attract the animals to the anode as is the case in freshwater, but this gradually changed when McBary (1956) stipulated that the theories used for freshwater could not be extrapolated to seawater. Additionally, pulsed current had to be used instead of direct current (DC), because of the high power demand needed due to the high conductivity of the seawater. From then on, the focus was put on a startle reaction of the target species, to make them leave the seafloor and enter the net. This would make it feasible to replace the heavy tickler chains or bobbins on conventional beam trawls with electrodes without loss in efficiency (Boonstra & de Groot, 1970).

In 1965, Mc Rae and French started experimenting with electric fields as an addition to the conventional stimulation in otter trawl nets, using the field to shock the fish upon their arrival at the net so that they would be immobilized and swept easily into the trawl. This multiplied the fishing effectiveness by a factor 1.5, 1.5, 2 and 4.4 for cod (*Gadus morhua* L.) and haddock (*Melannogrammus aeglefinus* L.), flatfish and whiting (*Merlangius merlangus* L.), respectively. In the U.S., Pease and Seidel (1967) developed a small electrotrawl (<3 V, 4-5 Hz) to catch shrimp during daylight and in clear water, when catching efficiency is normally very low. Depending on the substrate, the catches were 95-109% (muddy) to 50% (calcareous sand-shell) of the normal quantity caught at night. In Europe, this knowledge was adopted by a German group reporting a 30% catch increase (Unknown, 1969). In 1970 experiments were set up in the Netherlands with electrified nets (60 V, 2 ms, <5 Hz, 0.5 m) intended for brown shrimp (*Crangon crangon* L.). Besides the increase in catching efficiency at daytime, another advantage became apparent, namely the reduction of physical damage such as bruisings, ruptured fins and scale loss inflicted by tickler chains to immature flatfish by fishing with a less heavy gear (Boonstra & de Groot, 1970). In Belgium, Vanden Broucke (1972) obtained good results by means of a pulse generator (100 V, 2 Hz), dredging up 44% more shrimp and 250% more sole (on a small number of 39 individuals). In his quest to find suitable stimulations for other species, Stewart also investigated the effect on Norway lobsters (*Nephrops norvegicus* L.) (Stewart, 1972a & 1974). He found that electric pulses (1-5 Hz, 20-40 V $m^{-1}$) could stimulate emergence of these animals from their burrows in less than 5 s. Meanwhile the research on brown shrimp had continued as well, and Boonstra & de Groot (1974) found almost equal catch ratios for the electrified (60 V, 0.2 ms, 5

Hz) and the normal trawl. From then onwards, driven by the energy crises in the 1970's, priority was given to reducing the drag and consequently the fuel consumption of the more fuel intensive flatfish fishery.

The high sole catches obtained by Vanden Broucke (1972), triggered research on the adoption of electric pulses for sole to reduce the damage on immature flatfish and to economize the exploitation costs of the heavy tickler chains by replacing them by light electric ticklers (Boonstra & de Groot, 1970). The first experiments (Stewart, 1975a&c) suggested that the most efficient stimulation pattern for flatfish was a 1 s long burst of DC pulses at 20 Hz (50-60 V with electrodes 1 m apart), with 1 s delay between bursts. At higher frequencies a greater percentage of the flatfish remained tetanized on the bottom. It was also found that the 20 Hz PDC tended to preferentially stimulate larger flatfish, which was promising for a better selectivity. In the following years, studies with 3 to 4 m beams in the UK (Stewart, 1977 & 1978; Horton & Tumilty, 1983), Germany (Horn, 1976; Horn, 1977), the Netherlands (van Marlen, 1997) and Belgium (Vanden Broucke & Van Hee, 1967 & 1977) indicated indeed that light electrodes are an effective alternative for heavy tickler chains.

Despite the good progress that was made in the first decades, the challenge, especially on the technical side, was still enormous (Unknown, 1970; Stewart, 1971). It was very difficult to reproduce the results made with the small beam trawls in larger commercial 9 m beam trawls, as more electrodes and thus more power was required. The increased power demand, the water resistance of the voluminous pulse generators, the electrode connections in the water, the electrode material and the electric efficiency were all leading to an accumulation of technical difficulties and frequent malfunctioning (Boonstra, 1979). The low fishing speed and the lack of electric power, making it impossible to sufficiently stimulate sole, resulted in poor 50% less catch results (Boonstra, 1978). This hurdle was difficult to overcome at that time and hence markedly slowed down the further study and development of marine electrofishing. Half a decade later, a new generation of pulse generators enabled sufficiently high voltage peaks (Agricola, 1985). An increase in catch weight of 114% combined with a reduction of by-catch and benthos to almost 50% was achieved in Germany (Horn, 1982 & 1985). In the Netherlands, 45% and 65% more sole were caught during the day and during the night, respectively (van Marlen, 1997). In Belgium higher sole catches with an electrified otter trawl with less undersized fish and more fish above the minimum landing size were achieved (Delanghe, 1983; Delanghe & Vanden Broucke, 1983). The first commercial electric beam trawls were already commercially available in the Netherlands (Unknown, 1988a; Unknown, 1988b), when the German authorities did not allow electric fishing on a commercial basis in 1987 and the Dutch government followed their footsteps one year later. Later on 30 March 1988, the European Commission prohibited the use of electricity to catch marine organisms (EC nr 850/98, article 31: non-conventional fishery techniques). The main reason for this ban was the fear of further increasing catch efficiency in the beam trawling fleet which was under severe international criticism back then (van Marlen, 1997). Moreover, it became more and more difficult to obtain a cost-effective system with the falling prices of fuel (Unknown, 1988c). Additional hurdles were safety issues, malfunctioning or system breakdowns. This vulnerability, combined with the large investment and maintenance costs of an electrofishing device, hampered a successful introduction.

The reticence to electric fishing gradually changed when oil prices were sharply rising again some 20 years later, traditional beam trawls became less profitable, making the investment more economically feasible. At the same time the environmental impact issue became increasingly important. In the early 90's, new initiatives were taken in the Netherlands leading to a revival of the electrotrawls.

## Existing commercial electrotrawls

### *Flatfish*

Verburg Holland BV (taken over by Delmeco Group BV. since 2010) started in 1992 with the development of an electrified pulse beam trawl for flatfish, using a pulse to induce a cramp reaction instead of a startle reaction. In 1995, the first 4 m beam prototype was built and in 1997 a 7 m prototype was tested at sea. The results were to such an extent fortifying that the project was continued in cooperation with the Dutch ministry and fishery sector, leading to the up scaling to an operational 12 m beam fishing gear that was tested on the commercial vessel UK153 in 2004 (Figure 1.1a) (Van Stralen, 2005). The beam trawler TX-68 was the first commercial vessel fishing with this system in May 2009. Meanwhile another Dutch company, HFK engineering, had started its own developments, applying the pulse system on a new type of beam trawl, the so-called 'SumWing' trawl. In this gear, the cylindrical beam with trawl shoes is replaced by a wing-shaped foil with a runner at the centre (Figure 1.1b). This SumWing itself has less bottom contact compared to the conventional beam and due to its hydrodynamic wing-shape, it reduces the fuel consumption by some 10% (van Marlen *et al.,* 2014). The implementation of the pulse system to the 'SumWing' trawl is called 'Pulse Wing' and has a larger potential for the reduction of gear drag (50%), bottom impact and fuel consumption (van Marlen *et al.,* 2014). The beam trawler TX-36 was the first commercial vessel using this system at the end of 2009. The price for both systems is approximately € 300 000 for a large beam trawl and € 200 000 for a eurocutter.

The pulse systems receive electric power from the vessel by an additional cable that also provides communication between controls on board and the pulse generator on the fishing gear. Each electrode has a module that generates pulses independent of the other electrodes. This makes it possible to replace just one electrode module instead of the entire generator in case of malfunctioning. The pulse generator of the Delmeco electrotrawls fires the 25 electrodes attached to the beam on a mutual distance of ±0.42 m. The initial electrodes consisted of six different cupper conductors (ø26 mm, 0.18 m length) alternated with isolators and the total length of the electrode measures about 6 m (van Marlen *et al.,* 2014). The pulse wing on the other hand was initially rigged with 28 parallel 6 m long electrodes, at a mutual distance of 0.41 m. Each electrode is composed by 12 cupper conductors (ø33 mm, 0.125 m length) alternated with polyurethane isolators (van Marlen *et al.,* 2014). A detailed construction design of both systems can be found in van Marlen *et al.* (2011). Nowadays, fishermen make their own electrodes, which have led to much more diversity in electrode designs.

**Table 1.1**: Pulse characteristics(1) of the pulse beam on the TX-68 (Delmeco) and pulse wing on the TX-36 (HFK), both targeting flatfish (De Haan *et al.*, 2015), and the Hovercran system (Marelec) targeting shrimp (Verschueren *et al.*, 2012; personal communication with Verschueren November 2015).

| **Pulse System** | **Electric Power** | **Electrode distance** | **Peak Voltage** | **Frequency (2)** | **Duration** |
|---|---|---|---|---|---|
| (company) | (kW m$^{-1}$) | (m) | (V) | (Hz) | (µs) |
| Delmeco | 0.70 | 0.42 | 50 | 80 | 220 |
| HFK | 0.58 | 0.41 | 45 | 45-80 | 380 |
| Marelec | 0.13 | 0.67 | 60-100 | 4,5 | 500 |

(1) The pulse characteristics and parameters are explained on page 21.

(2) Frequency was measured as the number of pulses, not as the number of repeated pulse cycles, to avoid confusing between PBC and PAC. Therefore, values for PBC may be twice as high as given by de Haan *et al.* (2015)

**Figure 1.1**: The Delmeco pulse beam with beam and trawl shoes (A) and the HFK Pulse Wing with the wing and the runner in the centre (B).

The pulse characteristics are similar for both systems. They have a bipolar sinus and block pattern for the pulse beam and the pulse wing, respectively. The basic nominal design characteristics of the pulse systems are listed in Table 1.1. Note that the characteristics of more recent pulse trawls can be different. The electric parameter settings can also be adapted to the environmental conditions such as seawater temperature and salinity. These conditions may influence the conductivity or flatfish behaviour and thus the response to the electric pulse field (De Haan *et al.,* 2011). The movement of heavy tickler chains over and through the sea bed is normally responsible for 30% of the resistance of a trawl and they can penetrate up to 8 cm in the bottom (Paschen *et al.,* 2000). Replacing these tickler chains by electrodes hence greatly reduces the fuel costs and physical disruption of the seafloor. This less invasive impact on the seafloor also implies a reduced stimulation of the fish, which means a reduction of unwanted by-catch, which was clearly illustrated by the catch comparisons of van Marlen *et al.,* (2011). The net earnings (gross earnings – fuel costs) showed almost a duplication of efficiency for the TX-36 (186%) and large increase for the TX-68 (155%). However, the large investment and high maintenance costs of the electric gears are hereby not taken into account. The higher earnings result from the large savings in fuel consumption, caused by the slower towing speeds and the elimination of the drag caused by tickler chains.

## *Shrimp*

Encouraged by the rumours of successful application of electrotrawls in China and helped by the import of a Chinese prototype by the Belgian ship-owner Willy Versluys, the Belgian Institute for Agricultural and Fisheries Research (ILVO) started the development of an electrotrawl for brown shrimp in the late 1990's. The research of Polet *et al.* (2005 a & b) revealed that a half-sine pulse with a frequency of 5 Hz, a pulse duration of 500 µs and an electric field strength of approximately 30 V $m^{-1}$ gave the best result to startle brown shrimp (Figure 1.2). The low frequency and pulse duration make it possible to operate with a very low energy input of only 1 kWh per trawl (Verschueren & Polet, 2009). However, Marelec has been increasing the potential difference on its electrodes to over 100 V in recent years, which will also result in higher power demands (personal communication with Verschueren November 2015).

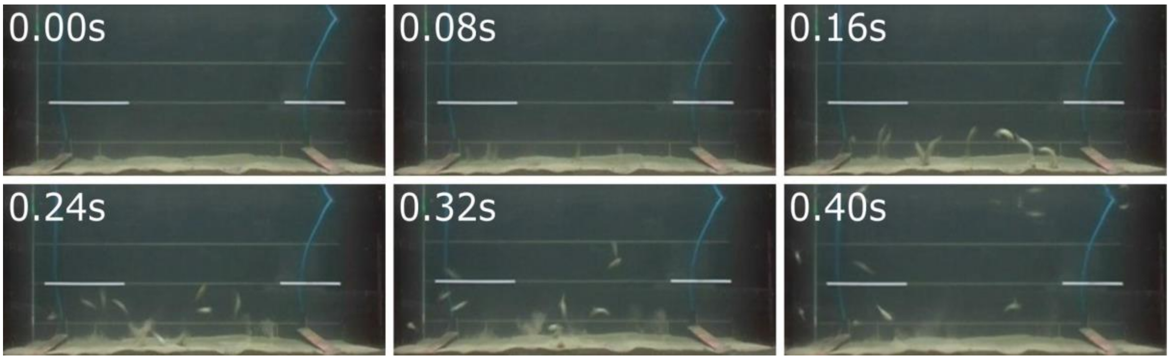


**Figure 1.2**: Exposure of brown shrimp to an electric field in the laboratory, the electric field is switched on at 0.05 s. The different points show startling reaction of the shrimp, forcing them to jump out of the sand (Verschueren & Polet, 2009).

Based on these findings, a commercial 8 m electrified shrimp beam trawl, the Hovercran, was developed in 2008 in cooperation with the Belgian company Marelec NV, and the University of Ghent (Figure 1.3). This electrotrawl consists of an on-board main control unit, connected with the pulley block at the top of the outrigger via a supply cable, which is hauled along with the fishing gear cable. The 12 electrodes (six cathodes + six anodes) form 11 electrode pairs and are fired alternatively by the pulse generator. The electrodes are 12 stainless steel cables (ø 12 mm, 3 m length) with a 10 $mm^2$ cupper core. The front 1.5 m is isolated and the last 1.5 m which is hanging horizontally above the seafloor, is an uninterrupted conductor. This is in contrast with the previous systems, where the electrodes were composed of alternating conductor and isolated parts (Verschueren *et al.,* 2012). The basic nominal design settings and pulse characteristics of the Hovercran are listed in Table 1.1.

**Figure 1.3**: The Hovercran with 8 m beam and trawl shoes. The cylinder fixed on the middle of the beam is the pulse generator.

In the original Hovercran concept, the trawl is meant to hover above the seafloor. Therefore the replacement of the bobbins by electrodes and an elevated footrope make it possible for non-target species to escape underneath the trawl. The targeted shrimp which are stimulated by the electric field to jump up in the water column are caught by the hovering trawl (Figure 1.4). With this setup, a similar catch weight of shrimp can be obtained and at the same time, bottom contact is reduced by 75%. An overall by-catch reduction of 35% results in cleaner catches, hereby improving the sieving process, the quality of the shrimp and reduces the workload of the crew. Moreover, the catch efficiency is less dependent on light and turbidity conditions. This contrasts with the traditional shrimp trawl where catch efficiency varies strongly with light intensity and turbidity of the seawater (Verschueren & Polet, 2009). Only a minor reduction in fuel consumption of 10 % was obtained with the Hovercran, because the drag resistance of this gear is mainly caused by the small mesh-sized net (Verschueren *et al.,* 2012).

In contrast to the original Hovercran configuration, rewarded with runner-up price of the WWF International Smart Gear Competition in 2009, the commercial vessels using this system in 2012 still use a bobbin rope. However, the number of bobbins is reduced from 32 to maximum 12 and the bobbin rope is straightened (Verschueren *et al.,* 2012). This way, the gain in selectivity and reduced bottom contact is less extreme, but the amount of shrimp caught has increased substantially. When electrodes are used in combination with a conventional trawl with 36 bobbins, much more shrimp can be caught, especially in clear water conditions (Verschueren *et al.,* 2012). The conversion of a conventional trawler to the Hovercran system costs approximately € 70 000.

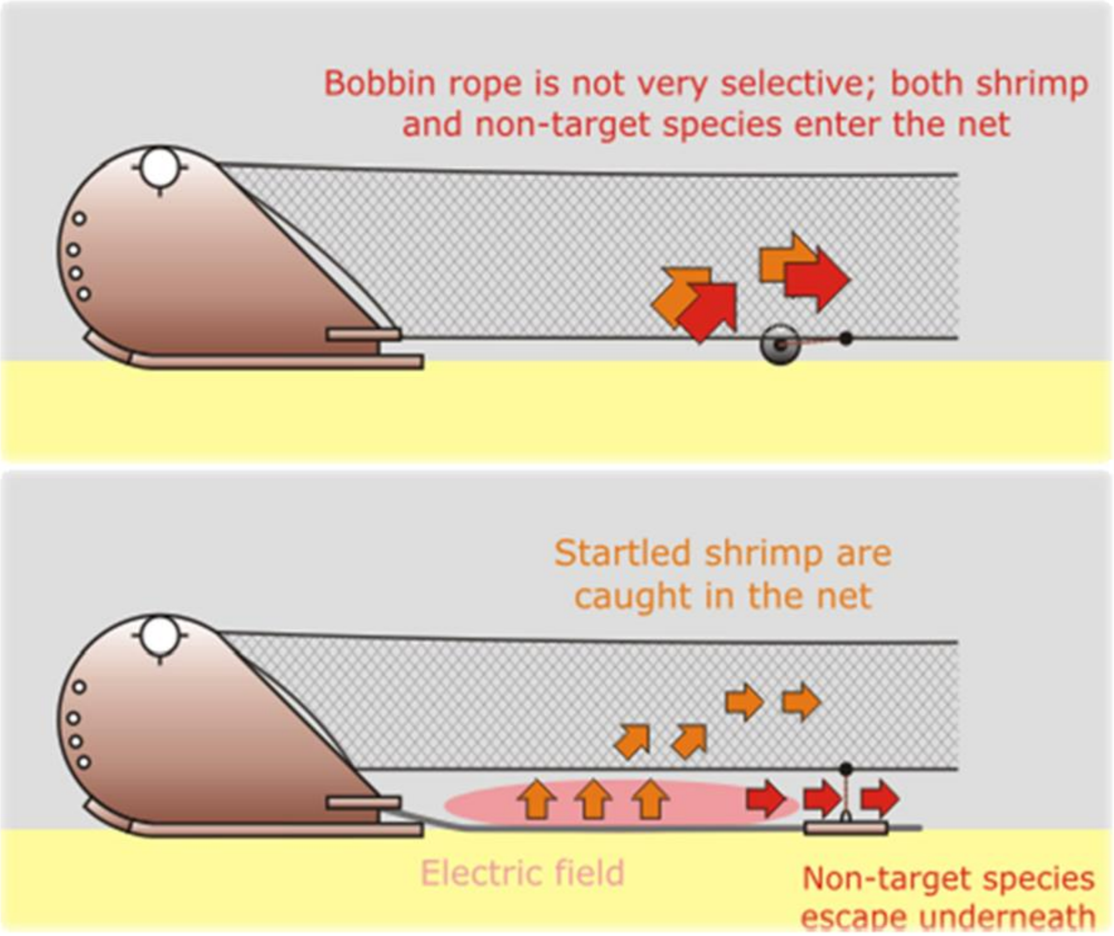


**Figure 1.4**: Schematic side view illustrating the basic principle of the HOVERCRAN (below) in comparison with the traditional catching technique (above); the bobbin rope has been replaced with electrodes, generating a specific electric field (Verschueren & Polet, 2009).

### *Razor clam*

Woolmer *et al.* (2011) experimentally designed and trailed methods to harvest razor clam (*Ensis spp.*, Pharidae) using electrical stimuli. This research group used 3 mild steel flat bar electrodes (30 x 8 x 3000 mm) on a separation distance of 0.6 m to produce maximal DC field strength of approximately 50 V $m^{-1}$. They demonstrated that electrofishing gear generating relatively low DC can be effectively used to stimulate the emergence of Ensis spp. from their burrows. No serious negative effects on the epifaunal and macrofaunal benthic community were detected during the month after a single pass of the electrodes. Therefore this is potentially a more environmentally benign alternative to existing hydraulic and toothed dredges (Woolmer *et al.*, 2011; Breen *et al.,* 2011).

## Changing political climate

The growing interest in the flatfish pulse trawl in the North Sea is mainly driven by the large reduction in fuel consumption. The significant reduction in discards and seafloor disturbance are extra commercial assets in the light of an increasing market demand for fish caught in a sustainable manner. These three characteristics are equally important benefits in terms of ecological sustainability. Altogether, these are convincing advantages compared to the traditional beam trawl fishery that is collapsing under the pressure of rising fuel prices and public and political criticism. These were valid arguments to question the ban on electrofishing (EC Reg nr 850/98, article 31: not-conventional fishery techniques).

Following its assessment, the International Council for the Exploration of the Sea (ICES, 2009) advised that while there were many positive aspects to the pulse trawl, several concerns about possible side effects on target and non-target species needed to be addressed before final conclusions could be drawn on the likely ecosystem effects of electrogears. The European Commission subsequently granted Member States a derogation of 5% of the fleet to use the pulse trawl on a restricted basis, provided attempts were made to address the concerns expressed by ICES. This permission however, only applies to the Southern part of the North Sea (ICES subarea IVb & IVc). This derogation has been renewed annually since 2007 and in the Netherlands all available licenses are being used, providing a total of 42 vessels at the ignition of our studies: 39 targeting flatfish and three targeting brown shrimp (rijksoverheid Nederland, 2011). By the end of 2012, the council of the European Union proposed to extend the derogation from 5 to 10% of the fleet, which means that the number of Dutch licenses can increase to 84 (European Council, 2012).

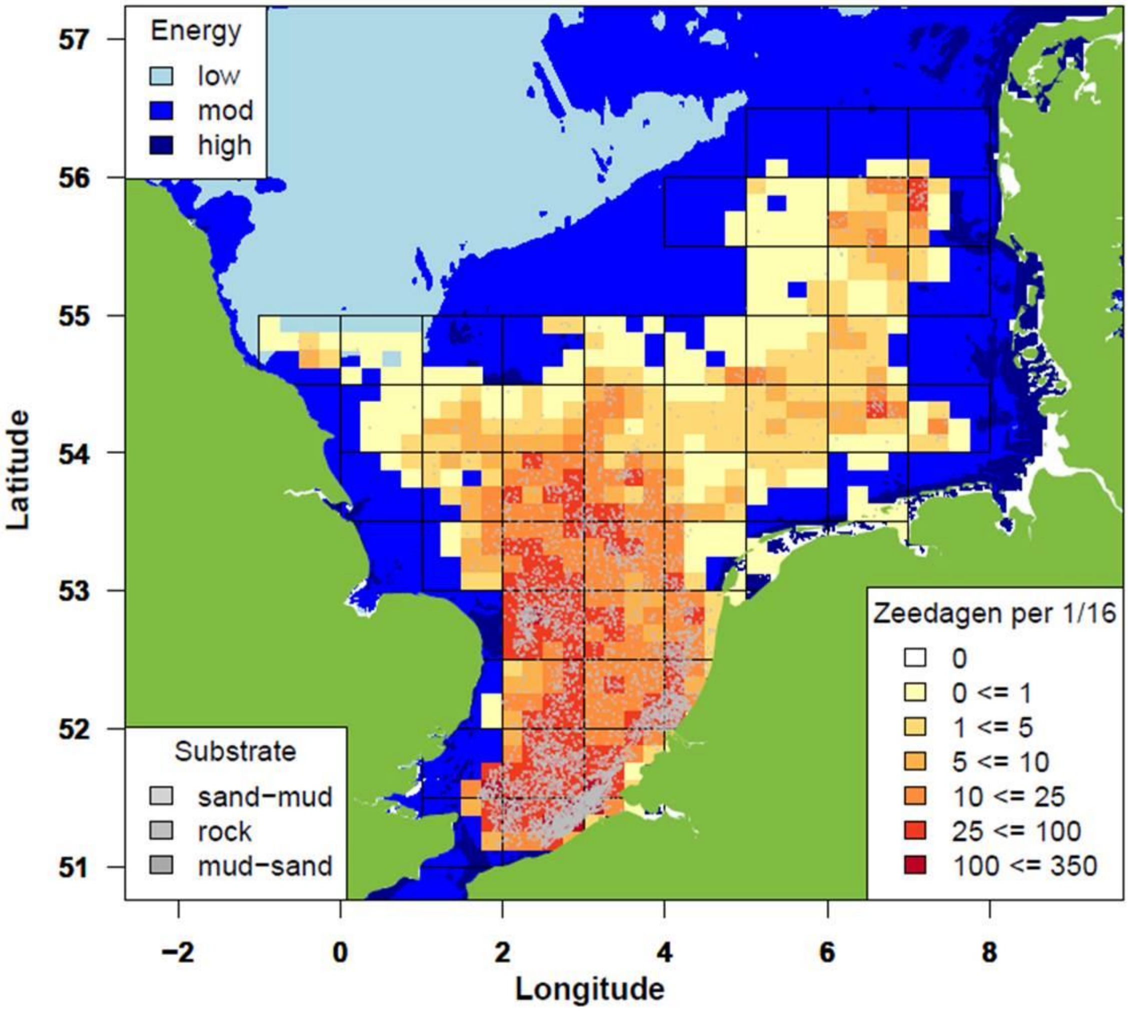


**Figure 1.5:** The fishing effort of electrotrawls in the North Sea in 2014 (reprint of IMARES report on pulse fishery distribution, with permission of IMARES and CVO)

By 2014, conventional beam trawls were already almost outcompeted by electrotrawls in the Netherlands: Dutch small (≤300 hp) and large (>300hp) electrotrawls were responsible for 91.6% and 97.2% of the total landings of sole by cutters respectively in the Netherlands (CVO, 2015). In other countries, the switch to electrotrawls is not so distinct yet, which is partially a consequence of the fact that the EU-derogation only covers the ICES subarea IVb & IVc. Belgian fishermen for exemple have only limited quota in this.

At this moment (end 2015), in total 93 vessels have already adopted this technique commercially, of which 1, 3, 9 and 80 have a Belgian, UK, German and Dutch licence, respectively. 65 of them are large electrotrawls (>300hp) and 28 are eurocutters (≤300 hp). One, 2 and 4 Belgian, German and Dutch eurocutters respectively are targeting brown shrimp using the Marelec system. All other vessels are electrotrawls targeting sole, of which 86% use the equipment of HFK-engineering and 14% the equipment of Delmeco. In 2014, The fishing effort (2014) of electrotrawls in the North Sea is illustrated in Figure 1.5.

## Working hypothesis of electric fields

### *Electric fields in water*

To be able to fully grasp the working principles and effects of electrofishing, a good understanding of the operation of electric fields in water is required, as is summarized below. The current (I) is defined as the movement of electrons provided by a power supply from a positively charged electrode (anode) to a negatively charged electrode (cathode). The difference in electric charge will create a potential difference (voltage [V]) over the two electrodes. Charged ions in the water will be attracted to the opposite charged electrode and in this way neutralize the potential difference over the two electrodes. This movement of charge in the water closes the loop of current driven by the power source. The more ions in the water, the higher its conductivity and the better its capacity to conduct electric current. This conductivity can vary strongly, depending on the water temperature, the water salinity and the organic matter content. The capacity of the power source to force electrons to go from one electrode to the other (power, [W]) is limited. Therefore, the potential difference over the two electrodes will be inversely proportional to the conductivity of the water, which is illustrated by the formula of electric power: $P = V^2 R^{-1}$, with P the power, V the potential difference and R the resistance, which is the inverse of conductivity. Indeed, when the conductivity is high as in sea water, the charge on the electrodes supplied by the power source will be easily neutralized and the potential difference will be small. Each potential difference over two electrodes induces an electric field in the water. This field is characterized by a field strength ($[V\ m^{-1}]$) which indicates the voltage gradient at a certain location in the medium between the electrodes.

Power sources can produce two types of current: direct current which is the movement of electric charges in one direction and Alternating Current (AC), which is a bipolar current flow. Both types can be applied with intervals and hence will generate pulses being called Pulsed Direct Current (PDC) or Pulsed Alternating Current (PAC), respectively (Figure 1.6). PDC and PAC are characterized by the frequency (F, [Hz]), which is the number of pulses per second, pulse duration (D, [μs]), pulse shape (S) and amplitude (A, [V]). The higher the potential difference on the electrodes, the higher the amplitude and the field strength will be.

The main advantage of the use of pulsed current is the limited power demand. The pulses help increasing field strengths by producing large bursts of peak power that are short in duration and intercalated with recovery periods in which the transformer and capacitor components store the energy required for the next burst (Novotny, 1990). Nevertheless this type of current is still able to attract and immobilize fish (Beaumont *et al*, 2002). PDC has in general frequencies of 50 to 100 Hz and are used at voltages of 100-400 V (Snyder, 2003).

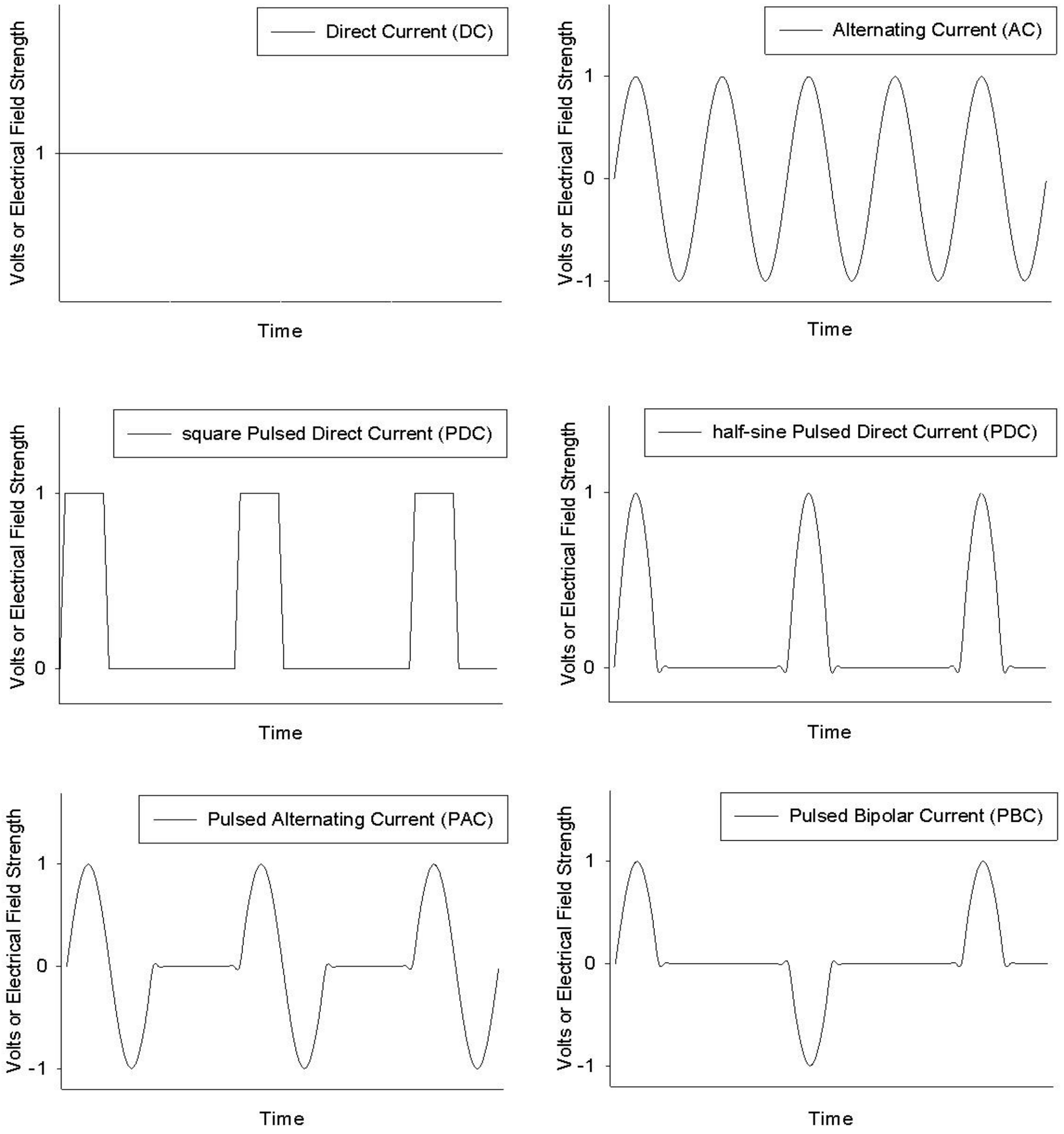


**Figure 1.6:** Different types of waveforms used in electrofishing.

## *Physiological effects of electric fields*

The physiological effect of electric fields on freshwater animals has been studied extensively. The data cited in this chapter concern freshwater organisms, as this might help to concede to the lack of knowledge on the effects in marine organisms. Largely, two different approaches are adopted to explain the reactions of freshwater fish to an electric field. First of all, different authors stated that direct nerve and/or muscle excitation is the major cause for the responses of the fishes in

DC fields (McBary, 1956; Lamarque, 1963; Vibert, 1963; Blancheteau, 1967). When the sensory nerves are being stimulated, the response is possibly of a reflex nature. If the motor nerves undergo stimulation, the response is probably due to their stimulation being transmitted directly to the muscles (McBary, 1956). Danyulite and Malyukina (1967) proved that locomotory activity and swimming are controlled by the spinal cord. When the spinal cord was cut, the reactions to electric fields stopped, while removal of skin receptors or the fish brain did not have any effect. Electric stimulation of the spinal cord can hence induce a muscle response in the fish. The reaction of organisms to PDC is more complicated, as very complex physiological processes such as chronaxies, spatial and temporal summations, synaptic delays, excitatory post-synaptic potential and polarity are involved (Lamarque, 1967). These neurological terms refer to the time gap between the onset of a pulse and the muscle contraction, the cumulative effect of stimulating multiple neurons at once or stimulating a neuron many times in succession and (de)polarization of the post-synaptic membranes which affect the action potential of neurons.

Secondly, Sharber & Black (1999) emphasized the similarities with the responses of other animals and humans subjected to electroconvulsive therapy. They stipulated that the various reactions can be seen as stages of epilepsy. Their insight originated from Delgado-Escueta *et al.* (1986), stating that epileptic events were describing the physiological response of animals, even at tissue and cellular levels, to a chemical, electric, or mechanical shock on the central nervous system. Once the central nervous system is overwhelmed by the stimulus, seizures occur (Penfield & Jasper, 1954). The onset of such epileptic events is frequently accompanied by myoclonic jerks, i.e. simultaneous contractions of the white muscle tissue on either side of the spine (Penfield & Jasper, 1954). This is important in relation to the occurrence of injuries (Sharber *et al.,* 1994) and will be discussed in more detail below.

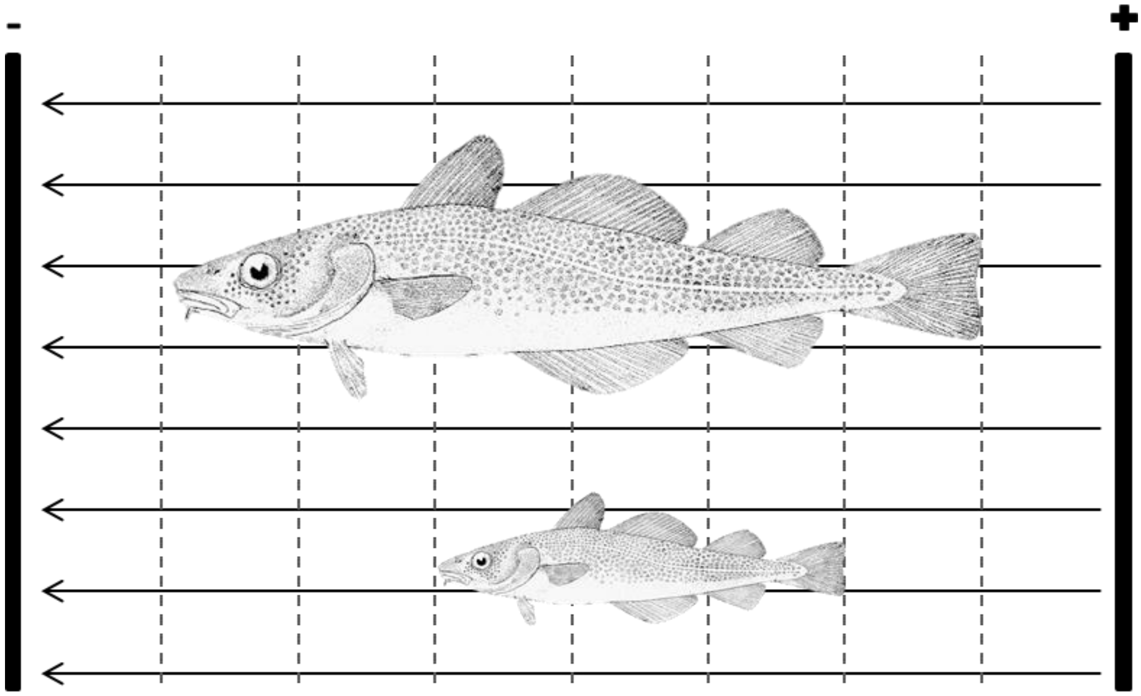


**Figure 1.7:** Draw of cod in a homogenous electric field. The heavy vertical lines represent two electrodes. The horizontal lines are the field lines, representing the current flow between the two electrodes. The dashed vertical lines are equipotentials, zones with the same potential. The larger the difference between two extremities of a fish (here: between head and tail), the higher the potential difference over its body and the stronger it is experiencing the electric field. Note that the orientation of the fish has a marked influence on the potential difference over its body.

Both approaches imply that minimal stimulus intensity is needed to exceed the threshold stimulation that causes a reaction of the fish, either to excite the nerve and muscle, or to give rise to an epileptic seizure. This elicits that the greatest effect will be observed when the potential difference is largest, namely when the longitudinal head-to-tail axis of the fish body is parallel to the field lines which is perpendicular to and between the electrodes, in case of plate electrodes that generate a uniform or homogenous electric field (Figure 1.7) (Snyder, 2003). Therefore, it is generally accepted that larger fish, with a larger potential difference over their body as illustrated in Figure 1.7, will show greater reaction (Adams *et al.,* 1972; Stewart, 1975; Emery, 1984; Dalbey *et al.*, 1996; Dolan & Miranda, 2003). McBary (1956) found that the relationship between fish length L and the voltage V required to produce a reaction was of the form V = aL + b, where a and b are constants. Therefore large fish still respond to lower field strengths than small fish. However, the sensitivity varies greatly between different species (Halsband, 1967).

At low frequencies, a PDC field will frighten the fish, that will try to swim away (startle reaction). This principle is used nowadays to catch brown shrimp (Polet *et al.,* 2005 a & b). Once the frequency exceeds a certain threshold value, usually around 20 Hz, the jerking movements of the muscle, induced by the electric pulses, are succeeding so fast that the muscles are continuously stimulated and remain contracted. This summation of many individual contractions may lead to a cramp and immobility (Snyder, 2003). This cramp reaction seems especially suitable for catching Dover sole because their powerful dorsal muscles make them bend in a U-form when going into cramp. It prevents the animal to escape and makes it easy to scoop the fish up with the ground rope (Van Stralen, 2005).

## Side-effects of electric fields

Snyder (2003) pointed out that electrofishing involves a very dynamic, complex, and often misunderstood mix of physics, physiology, and behaviour. The determination of possible harmful effects on fish is therefore a giant task. Because most fundamental research about the harmful effects on fish was done in freshwater species, a selection was made by the author with the intention to give an image of the harmful effects that can possibly, but not necessarily, be expected for saltwater species exposed to the PDC used in electrotrawls.

Although the freshwater research offers a lot of data, one always has to remember that it is incorrect to extrapolate the findings observed in freshwater research to seawater because there are large differences in sensitivity amongst different species (Halsband, 1967; Emery 1984) and the distribution of the electric field in and around the fish is completely different in freshwater compared to seawater. This is reflected in the applied exposure time which is at least 10 times longer in freshwater than in seawater (only 1-2 s), the applied voltage which is often 2-6 times higher in freshwater, and the pulse type chosen. Indeed, AC is sometimes used in seawater, which is more harmful to fish than PDC (Snyder, 2003). Nevertheless, the data generated from studies involving freshwater fish species may give a better insight in certain trends of possible effects in case information on marine species is lacking. A brief overview of fresh water data is given per fish family in Table 1.2.

**Table 1.2**: Concise overview of electrofishing effects reported in fresh water fish per family. The data is a summary of the compendium in the review of Snyder (2003a). Note that these data are obtained under very versatile experimental conditions, both in the field as in the laboratory.

| Family | # Species studied | Mortality | Haemorrhages | Spinal injuries |
|---|---|---|---|---|
| Cyprinidae | 21 | 0% | 0-27% | 0-15% |
| Catastomidae | 10 | 0% | 0-50% | 0-18% |
| Ictaluridae | 1 | 0% | ? | 60% |
| Esocidae | 1 | 0-0,2% | 0-19% | 5-33% |
| Salmonidae | 12 | 0-93% | 0-91% | 0-86% |
| Gadidae | 1 | 0-50% | ? | ? |
| Cottidae | 1 | 0-60% | ? | ? |
| Centrarchidae | 8 | 0-94% | 0-14% | 0-33% |
| Percidae | 4 | 0-95% | 0% | 0-40% |
| Sciaenidae | 1 | 0% | 0% | 0% |

## *Harmful effects on freshwater fish species*

The most reported harmful effects of PDC are spinal injuries and associated haemorrhages as observed in rainbow trout (*Oncorhynchus mykiss* W.), documented in up to 50% of fish examined internally (Sharber & Carothers, 1988).

In some cases 29-100% of the exposed fish are affected, with even the lowest voltages and frequencies causing a substantial amount of internal haemorrhages (Schreer *et al.* 2004). These injuries are most probably induced by myoclonic jerks (Sharber *et al.,* 1994; Fink, 1979) provoked by pulsating changes in field intensity, for example when the current is switched on and off. As each pulse can be seen as such an on-off switch, the frequency of PDC appears to be a primary factor affecting the incidence of spinal injuries and may be a significant factor in electrofishing mortalities (Sharber *et al.,* 1994; Snyder, 2003). The link between spinal injuries and mortality was contradicted for warmwater species such as centrarchids. Crappies showed spinal injuries at 5, 60 and 110 Hz but while haemorrhaging was higher at 60 and 110 Hz, mortality was only seen at 5 Hz (Dolan *et al.*, 2002). This was confirmed by Miranda & Kidwell (2010), who concluded that the mortality of the warm freshwater non-game test species was not related to gross-scale injuries because similar or worse haemorrhages and spinal injury were seen in fish that survived electroshock and those that died. This finding suggests that the mechanisms causing physical injuries are not the same as the mechanisms that cause immediate mortality. Besides, Dolan & Miranda (2004) found higher injury and mortality when pulses with a lower duty cycle were used in other centrarchids like bluegill sunfish (*Lepomis macrochirus)* and largemouth bass (*Micropterus salmoides)*, while the opposite was observed in trout. Obviously, there appears to be a fundamental difference in the effects on salmonids such as trout and warmwater species such as centrarchids, which might be due to their physiological or morphological differences: several warmwater fish species have fewer and larger vertebrae which are more resistant to injury whereas trout have many small vertebrae surrounded by a rather large muscle mass.

Electric shocks also have some effect on cardiac functions. Although Kolz and Reynolds (1990) stated that cardiac arrest is seldom a factor in fish mortality, Schreer *et al.* (2004) observed cardiac arrest in rainbow trout. This lasted for the duration of the shock, immediately followed by a period of arrhythmia of a few seconds to several minutes after the shock. An exposure (2 ms, 30 Hz, 100 V) of rainbow trout during 2 s, which is comparable to the pulses used in electrotrawls, resulted in a cardiac arrest of 6 s, a cardiac recovery time of 40 min for the heart rate (108% intensity), a cardiac recovery time of 120 min for the cardiac output (165% intensity) and stroke volume (193%), while the behavioural recovery time was only a couple of minutes. With regards to cardiac functions, shock duration appeared to be the major factor, while higher voltages and frequencies result in longer recovery times (Schreer *et al.* 2004). These values are in the same range as the results Emery (1984) obtained when recording physiological changes during exposure to electric current. An increase in oxygen consumption ranging from 110 to 150% depending on the current was observed, with a recovery time of 30-120 min. According to Emery (1984), this is possibly the result of lactic acid accumulation due to the rapid muscular contractions induced by the electricity. While most fish will recover from this build-up of lactic acid within 4-12 hours, some fish will never recover resulting in delayed mortality.

Long term effects of electrofishing on rainbow trout were examined by Dalbey *et al.* (1996) with some remarkable results: fish with intermediate or severe injuries (28% of total) showed a significantly reduced growth and condition, and 1 year after exposure (10 ms, 60 Hz, 200-400 V), the initial spinal injuries had increased with 60%. This was in contrast with the rapid physiological and behavioural recovery. Moreover, no proof was found that the pulse form or the initial injury had an effect on the long term survival of the fish.

Finally, the impact on early life stages is also of major concern. Despite several investigators reporting no evidence of harmful effects (Halsband, 1967; Halsband & Halsband, 1984; Walker *et al.,* 1994), others showed that exposure of egg carrying fish to electric fields can cause significant damage or premature expulsion of gametes and sometimes reduced viability of subsequently fertilized eggs (Marriott, 1973; Roach, 1996; Muth & Ruppert, 1996). The survival of embryos on or in the substrate was also affected, particularly when exposure happened between precleavage stages and eyed-egg stages (Godfrey, 1957; Lamarque, 1990). This early stage of development was also most vulnerable when exposed to mechanical shocks (Kolz & Reynolds, 1990). Exposure of recently hatched larvae might not cause significant mortality but can reduce growth rates for at least a few weeks, although significant differences in growth were not detected until 21 days after treatment (Muth and Ruppert, 1997). According to Maxfield *et al.* (1971), there was no long-term effect on survival and growth of yearling rainbow trout. The most critical parameters affecting embryos and larvae appear to be the field intensity and duration of exposure (Dwyer *et al.,* 1993; Dwyer & Fredenberg, 1991). This data set seems to indicate that the sensitivity of early life stages is decreasing as their development proceeds.

## *Harmful effects on salt water fish species*

The knowledge of possible negative or harmful effects on marine organisms is scarce (Table 1.3). Cod is encountered most frequently in research because it appeared sensitive during sea trials with 4 out of 45 fish caught suffering from spinal fractures (van Marlen, 2011). Small juvenile cod fish (0.12 - 0.16 m), exposed to high field strengths of 250-300 V $m^{-1}$, all survived with post mortem examination not revealing vertebral injury nor haemorrhage (De Haan *et al.,* 2011). On the contrary, 50-70% of large cod (0.41-0.55 m) exposed to field strengths of 40-100 V $m^{-1}$ showed vertebral injuries. A reduction of injuries was noted when using increasing pulse frequencies higher than 80 Hz (De Haan *et al.,* 2011). De Haan *et al.* (2009b) demonstrated that the position of the fish relative to the conductors of the electrode was a decisive factor towards the effects noted. Indeed, cod exposed outside the distance range of 0.4 m from the electrodes, representing fish in the region just outside the trawl, did not react to the exposure and exhibited normal feeding behaviour. However, negative effects occurred when the fish were located in the near distance range of 0.1-0.2 m from the electrode: about 20% died shortly after exposure and 30% by day 14 following exposure. In total, 45% of the fish exposed to the near field had injuries, while no lesions were found in fish exposed at more than 0.2 m of the electrode. The bone fractures were located ventral to the third dorsal fin, which was explained by the authors as due to strong muscle contractions during exposure. Fish exposed at 0.2-0.3 m of the electrodes during exposure, displayed milder contractions without getting injured and responded well to feeding cycles. The high peaks in field intensity near the electrodes proved to be a major factor determining possible harmful effects.

Besides cod, dogfish (*Scyliorhinus canicula* L.), was included in the study. This electro-sensitive fish uses electroreceptors to locate its prey, based on the very low bio-electric fields produced by every living organism (Kalmijn 1966, 1982; Tricas, 2001). This might render these animals vulnerable to electric pulses. De Haan *et al.* (2009a) exposed three groups of 16 dogfishes with similar lengths (0.3 – 0.65 m) in the same experimental set-up as described for cod, but each fish was exposed four times in a row for 1 s. No mortality, macroscopic lesions or aberrant feeding behaviour were observed in the first nine months after exposure.

A first series of experiments to examine the effect of electric pulses on benthic invertebrates was done by Smaal & Brummelhuis (2005). They exposed 19 different species belonging to molluscs, echinoderms, crustaceans and polychaetes to electric pulses with amplitude that was two times higher and an exposure of eight times longer than the settings used in practice on commercial vessels. Reactions during exposure were minor or negligible and the survival after three weeks did not deviate from the control group. Van Marlen *et al.* (2009) exposed a selection of six benthic invertebrates to three subsequent 1 s bursts at different distances from the electrode, ranging from 0.1 to 0.4 m. For the ragworm (*Allita Virens* S.), European green crab (*Carcinus maenas* L.) and the razor clam (*Ensis directus* L.), a lower survival of maximum 7% was observed, while for common prawn (*Palaemon serratus* L.), subtruncate surf clam (*Spisula solidissima* L.) and common starfish (*Asterias rubens* L.) no significant effects on survival were found. The food intake was only significantly lower (10-13%) for the European green crab. All other species did not deviate from the control group in food intake or behaviour after exposure. This made the authors conclude that "it is therefore plausible that the effects of pulse beam trawling, as stimulated in this study, are far smaller than the effects of conventional beam trawling".

**Table 1.3**: Summary of side-effects in marine fish species resulting from electric exposures. All electrotrawl results refer to exposures to the cramp pulse for sole at ±80 Hz PBC/PAC (1,2-3,1% duty cycle). The stunning data were obtained in homogenous laboratory experiments with 50 Hz AC (100% duty cycle).

| | Species | Size (cm) | Haemorrhages | Spinal injuries | Reference |
|---|---|---|---|---|---|
| electrotrawl | whiting(1) | 27-38 | ? | 2% | van Marlen *et al.*, 2014 |
| | cod(1) | 20-84 | ? | 9% | van Marlen *et al.*, 2014 |
| | cod(2) | 41-53 | 0% | 0% | de Haan *et al.*, 2009b |
| | cod(3) | 44-55 | 45% | 40% | de Haan *et al.*, 2009b |
| | cod(2) | 12-16 | 0% | 0% | de Haan *et al.*, 2011 |
| | cod(3) | 34-56 | 0% | 0% | de Haan *et al.*, 2011 |
| | cod(3) | 34-56 | ±55-75% | 50-70% | de Haan *et al.*, 2011 |
| | dogfish(3) | 30-65 | ? | 0% | de Haan *et al.*, 2009a |
| stunning | herring | 29 ± 2 | ? | 60% | Nordgreen *et al.*, 2008 |
| | salmon | 50-70 | 0-73% | 0-46% | Roth *et al.*, 2003 |
| | salmon | 65 ± 6 | 20-90% | 0-40% | Roth *et al.*, 2004 |
| | pollock | 46 ± 5 | 60-80% | 0-40% | Roth *et al.*, 2004 |

(1)fish exposed during commercial fishing; (2)fish exposed above or far from the electrodes; (3)fish exposed near the electrodes

# Challenges and opportunities for the future

Electrotrawls may consitute a substantial improvement towards sustainability compared to the traditional beam trawls used to target flatfish and shrimp. The most impressive step forward for the flatfish fishery is undoubtedly the large savings in fuel consumption, up to 60% (van Marlen *et al.,* 2014), leading to a substantial increase in profit. Regarding environmental impact, all pulse trawls obtain significant discard reductions. Additionally, the impact on the seabed may be strongly reduced. The Hovercran has the potential to reduce the bottom contact with 75%, provided all bobbins are removed (Verschueren & Polet, 2009). In practice, not all bobbins are removed, but still the bottom contact is reduced by at least 30%. Still, this constitutes a marked improvement and further optimization aimed at further reducing sea bed contact is on-going and should be a major focus point. It should be stated that in the case of the flatfish electrotrawls, the reduction in bottom contact is limited, because the footrope is still towed over the complete width of the trawl. However, the intensity of the seafloor impact is lowered as the tickler chains, which can normally penetrate up to 0.08 m in the sediment (Paschen *et al.,* 2000), are removed. Moreover, the innovation has not stopped with the introduction of the pulse trawl. The wider commercial application in the North Sea will undoubtedly boost innovation and its selectivity can be improved even more in combination with escape windows and sorting grids.

These reasons indicate that electrotrawls may pose a valuable alternative for the conventional beam trawls. However, to be able to rectify the above statement, a vast amount research is still to be done on the unwanted side-effects and how these can be mitigated, and on the further reduction of the discards. The various research items in these areas that need to be addressed are discussed below.

## *Unwanted side-effects*

### A. Impact of pulse parameters

In general, pulse **frequency** rather than high voltage gradients appears to be the primary cause of spinal injuries and haemorrhages. This is clearly demonstrated in freshwater by Sharber *et al.* (1994), showing only 3% of the exposed fish were injured at low frequency (15 Hz), but 24% and even 43% of the fish were injured at moderate frequencies of 30 and 60 Hz, respectively. Snyder (2003) added the comment that lower frequencies can still cause injuries if the voltage is raised above a certain threshold, which was confirmed by Schreer *et al.* (2004). This trend seems to be valid in seawater as well: while Vercauteren *et al.* (2012) did not see spinal injuries in cod at low frequencies, 7 to 70% spinal injuries were reported at moderate frequencies, depending on the voltage gradients (De Haan *et al.,* 2009b & 2011; van Marlen, 2011). The reduction of injuries at frequencies > 80 Hz, to no visible injuries at 180 Hz, as observed by De Haan *et al.* (2011), seems to disprove this. However, during these experiments the duty cycle (percentage of time the current is flowing) was kept constant. This means that the pulse duration decreased when the frequencies increased, resulting in very narrow peaks at high frequencies that were likely too short to induce muscle contraction. This phenomenon was also observed by Bird & Cowx (1993). These researchers demonstrated that the frequency and duty cycle of PDC had strong interactive effects and that threshold field strengths for perception and attraction responses increased with frequency at low (10%) duty cycles. As De Haan *et al.* (2011) kept the field strength constant, the amount of pulse energy might have become too low at higher frequencies and lower pulse durations to induce reactions and injuries. A possible alternative improvement to reduce the spinal injuries without losing catch efficiency was given by Sharber *et al.* (1994). It was determined that a pulse train of 15 Hz, 15 bursts of several quick successive pulses in 1 second, with the same energy content as pulses of 60 Hz induced similar effects on the fish but caused fewer injuries. Hence, the use of pulse trains might offer a promising alternative. However, the effect on other marine fishes should be examined thoroughly as well, because there might be large differences in reaction between species as proven in freshwater research with salmonids and centrarchids.

The **field strength** also seems to play a primary role in the amount of injury and mortality observed. The higher this parameter, the stronger the voltage gradient in the water, the larger the difference in electrical potential experienced by the fish and the risk for injuries. This was clearly illustrated by the experiments of De Haan *et al.* (2011). The majority of cod exposed to higher field strengths (i.e. near the electrode) showed injuries, whereas effects were absent at lower field strengths (0.4 m away from the electrode). Besides, large adult cod showed much more injuries than small juvenile cod, even though the juveniles were exposed to much higher field strengths. In both cases a higher potential difference over the fish body elicits a stronger reaction of the fish. Another, additional, not experimentally tested hypothesis for this phenomenon was made by Stewart (1967, as cited by Lamarque, 1990), who suggested that spawning fish, particularly salmon, may be especially susceptible to spinal injuries due to skeletal decalcification and weakened or brittle bones. To the author's opinion, another factor can play a role as well: different stages of calcification, from cartilage in yearlings to bone in old adult fish, can affect the sensitivity of spinal structures for the strong contractions during myoclonic jerks observed during exposure. Further research to clarify this effect is definitely needed.

The **exposure time** is mentioned by different authors (Schreer *et al.,* 1994; Emery, 1984) as determining parameter regarding cardiac arrests. Schreer *et al.* (1994) reported recovery times of 40 min and 120 min for the heart rate and cardiac recovery time, respectively after a 2 s exposure, with a pulse duration that was up to eight times longer than applied in electrotrawls (2000 µs versus 250 µs).

Similar recovery times are seen in other stress situations: 40 min after noise disturbance (Graham & Cooke, 2008) and up to 210 min after angling (Schreer *et al.,* 2001). Although this clearly indicates an effect, fish exposed to the capture process in beam trawls will also experience stress. Important to note though, is the fact that the cardiac recovery time was 10-100 times longer than the behavioural recovery time of only a few minutes. The same was stated by Dalbey *et al.* (1996), who found that the rapid physiological and behavioural recovery contrasts with reduced long term growth and conditions and increasing injuries. This indicates that behaviour cannot be used as the only parameter when assessing the impact of electric pulses on an animal and that various parameters need to be included.

Finally, the **pulse type** and **pulse shape** are two parameters which can influence the reaction of the fish to electric pulses. However, they have not yet been thoroughly examined. Concerning the pulse type, it is generally accepted that AC is the most and DC the least harmful, with PDC in between (McBary, 1956; Sharber, 1994; Dalbey *et al.*, 1996). This suggests that the Pulsed Alternating Current (PAC) and the Pulsed Bipolar Current (PBC) used in the electrotrawls for flatfish might be more harmful than PDC used in the Hovercran, but no direct comparison between bipolar pulses and PDC has been made yet. De Haan *et al.* (2011) found that a time delay between the positive and negative parts of the bipolar pulses seems to contribute to injury, although not in a significant way. Although most authors agree that quarter sinus waves are the most harmful (Sharber *et al.,* 1994; Bird & Cowx, 1993), it is uncertain whether an exponential or a square bloc wave is the best one to use.

### B. Effects on growth and development

Dalbey *et al.* (1996) observed reduced **long term effects** on growth and condition and an increasing number of injuries in rainbow trout. Although the exposure time was more than 10 times higher than what is encountered in electrotrawls, this indicates that a long term effect cannot be excluded and that the severity of injuries might even increase in time. Furthermore, the number and severity of injuries was positively related with the length of the fish (Dalbey *et al.*, 1996). Large commercially important fish will normally be caught after exposure and slaughtered immediately. Only in discarded specimens such as larger non-commercial or undersized commercial species long term effects are relevant. As such, the effect on electro-sensitive species should be further investigated. Despite the reassuring results of De Haan *et al.* (2009a), who found no evidence of aberrant feeding behaviour, this does not prove that the electro-sensitive organs of the fish are undamaged. Indeed, in their natural habitat, these fish fully depend on these organs to detect the very low electric fields produced by preys situated in the bottom. This is not the case in captivity, where they can easily find their daily meal in the clean survival tanks without having to resort to their electro-sensitive organs.

The reported effects on **early life stages** are contradictory and could reflect the differences in species sensitivity. Nevertheless, according to Snyder (2004), a sufficient number of indications were found to consider that freshwater electrofishing over spawning grounds can harm embryos. For several reasons it can be assumed that this effect will be more moderate in seawater. At first, the most critical parameters affecting embryos and larvae appeared to be the field intensity and duration of exposure (Dwyer *et al.,* 1993; Dwyer & Fredenberg, 1991). As mentioned before, these parameters have a much lower value in seawater. Secondly, the effect on mature fish is of minor importance for commercial species, since they are normally larger than the minimal landing size and will be landed after being caught. A last factor mitigating the risk on exposed embryos and larvae is their distribution in the water column. Whereas the electric field covers the whole water column in freshwater, the electric field is limited to the net opening in marine electric fishing. According to the results of Conway *et al.*

(1997) less than 12% of the eggs and larvae of sprat (*Sprattus sprattus* L.), dragonet (*Callionymus spp.*) and dab (*Limanda limanda* L.) were found in the 5 m water column zone above the seafloor. Furthermore also the area of the North Sea being trawled is limited. This implicates that the chance for exposure of eggs and larvae is very small. However, this obviously will need to be re-evaluated when electrotrawls are used in shallow spawning areas. Hence, further research on the effect of electric fields on the early life stages of marine species spawning in these shallow zones is strongly recommended.

### C. Effect on the sediment

A last aspect that should be investigated in the future is the possible electrolysis effect of the sediment. The high peaks in current might possibly induce the formation of toxic metabolites or release of heavy metals, definitely in substrates rich in organic matter and bounded metals (Alvarez-Iglesias & Rubio, 2009). No research whatsoever has been performed on this topic, but in view of the fact that a fan of chemical reactions is possible, this particular aspect also deserves further examination.

## *Reduction of discards & consequences*

There are four major **reasons explaining discard reductions**: (i) larger animals will react more easily on a stimulus, induced by a certain electric field strength than smaller ones (McBary, 1956; Adams *et al.* 1972; Emery, 1984; Dolan & Miranda, 2003), which explains the decrease in the amount of undersized fish caught, (ii) the electric pulses stimulate the target species and most invertebrates will hardly be stimulated by the field (Smaal & Brummelhuis, 2005; van Marlen *et al.*, 2009), (iii) the less intensive bottom contact prevents a part of the animals from being shovelled from the bottom (flatfish) or give the animals more chance to escape between the bobbins (shrimp), (iv) the reduced towing speed of electrotrawls results in a smaller fished surface, so fewer animals will be encountered and (v) this reduced towing speed also increases the chance of escape for the animal after it has entered the net.

The reduction in discards is an ecological improvement that all electrotrawls used in the North Sea have in common. The Hovercran shrimp pulse trawl showed a discard reduction of 35% with equal or increased shrimp catches (Verschueren *et al.*, 2012). For the flatfish pulse trawls, van Marlen *et al.* (2011) reported a 30-50% and 48-73% discards reduction measured in kg $h^{-1}$, for fish and benthos respectively but this goes together with a loss of commercially sized sole of 13-22%. However, the further development of this technique has led to better sole catches compared to the conventional beam trawls. More recent and elaborate scale catch comparisons showed a 10-20% increase in sole catches (kg $h^{-1}$), while reduced bottom contact results in a 16-42% reduction of benthos in numbers (Rasenberg *et al.*, 2013), which means a further decrease of discards per unit of fish landed.

In the pulse trawl for shrimp, the by-catch can be further reduced by raising the footrope (Verschueren & Polet, 2009). Consequently, also more shrimp tend to escape beneath the ground rope. This means that the height of the footrope will always be a trade-off between acceptable shrimp catches and sufficient by-catch reduction, offering fishery management two possible directions for ecological improvement with constant shrimp landings. The first is the Hovercran like it is used on four commercial vessels today, without raised footrope and with (a reduced number of) bobbins. The benefit in bottom contact and by-catch will be limited, but more shrimp will be caught. If total allowable catches for shrimp would be restricted with the wider introduction of the pulse trawl, the hours trawled would decrease due to the increased catching efficiency. Fewer hours trawled also means less surface dragged, less by-catch produced and less fuel consumed. The second scenario is the one with a bobbin-free and raised footrope. In this case the shrimp catches will not increase, but the seafloor disturbance and by-catch will be reduced drastically. The economic advantage for the fisherman would be an easier access to vulnerable fishing grounds and an easier access to the market of sustainably caught fish.

Last but not least, the reduction in discards of commercial species may also have large economic implications. Cappell (2001) calculated that 70% of the total landing value of the Dutch beam trawl fleet was lost due to this discarding. A saving of 30% in fish discards would imply a substantial reduction of the direct loss of potential income. Based on the landings of this fleet in 2011 (€ 210 Million), one can calculate that a saving of 30% in fish discards would lead to an annual increase of landing value of the Dutch fleet of several ten Millions.

### *Altered fishing effort*

The shift to pulse fishery on flatfish will definitely affect the accessibility of new fishing grounds. Muddy fishing grounds, however, that could previously not be fished with tickler chains can more easily be fished with pulse trawls. As such an extension of fishing grounds may occur for some fishing fleets of which the consequences should be carefully monitored, as pulse fishers for example could shift their fishing activity to the territory of passive fishers.

The pulse trawl for shrimp may result in increased catch efficiencies. As there are no quota or total allowable catches for shrimp, this may lead to increased fishing efforts which have to be approached with care. Yu *et al.* (2007) describe how the use of electrotrawls on several shrimp species in inshore waters of the East China Sea, has led to a large decrease of the biomass due to increased catch rates and total landings. To compensate for the reduction in catch rates due to the overfishing, electric output was increased to catch also undersized shrimp, resulting in complete biomass depletion until electrofishing was banned in 2001. The pulse technology used in the brown shrimp fishery in the North Sea increases the catching efficiency of the trawl significantly. Overfishing of the stock in the North Sea is, however, unlikely for multiple reasons. First, the demand on brown shrimp is limited and characterized by a price flexibility of about one (Revill *et al.*, 1999). An increased landing of 1% will thus make the price drop with 1%, so strongly increased landings are not beneficial to the fisherman. Secondly, there is no incentive of the fishermen to catch undersized shrimp, as there is a minimum size for shrimp to be sold. Finally, the electric output of the Hovercran equipment is limited and researchers of ILVO even proved that catching efficiencies were highest at 80% of the output (Verschueren *et al.,* 2012), so manipulating the output will not result in higher catches. However, this cannot be explained by the author and additional catch comparisons should be performed to further confirm this (Personal Communication with Verschueren, B.).

Nevertheless good management measures will be necessary to guarantee a positive application of this innovative technology, both in flatfish as shrimp fishery. As suggested by Yu *et al.* (2007), this management should include (i) certification procedures for device manufacturers and maintenance agents to avoid illegal production, trade and use, (ii) introduction of tamper-proof key settings for the output power parameters, (iii) introduction of specialized equipment to monitor the electric parameters in the field, and (iv) strict control of total fishing effort and total allowable catch.

# Conclusion

Electrotrawls are superior to conventional trawls regarding different aspects, including ecological impact on the North Sea (less bottom impact), management of commercial fishing stocks (less discards) and carbon footprint (reduction of fuel consumption). At the same time this alternative technique is more beneficial for the fishermen, because their earnings can be increased drastically and because they can catch more and independent of the time of the day and weather. Therefore electric pulse fishery seems to be the most promising alternative meeting both the fisherman's aspirations and the need for ecological progress.

Unfortunately, not all possible negative side-effects can be excluded yet. Although various studies elucidating the effects of electric fields on fish have been performed, some major gaps of knowledge still remain and need to be investigated:

- Is there a safe range of pulse parameters that allow application without (significant) side-effects for any marine organisms?
- What are the differences in sensitivity between different (in)vertebrate marine species and what is the effect on designing electrotrawls and setting the protocols?
- What are the effects on early life stages of marine species spawning in shallow zones where electrified trawls might be used?
- What is the long term effect on small non-commercial species or undersized species that can be exposed repeatedly?
- What is the effect on the electro-sensitive organs of electro-sensitive fishes?
- Is there an electrolysis effect of the substrate and water column resulting in the formation of toxic metabolites?


C-Power

# CHAPTER 2

# AIMS AND OUTLINE OF THE STUDY

# General Aims

The pulse fishery covers two types of techniques, each using a specific pulse. A minority of the electrotrawls target brown shrimp and aim for a startle response that makes shrimp jump out of the sediment. The majority target sole and use an electric stimulus to induce a cramp reaction in this flatfish. Both types are very successful and promising from both ecological and economical point of view. However, restricting and/or avoiding possible side-effect of electric pulses on marine benthic animals is warranted from ecological and ethical point of view and because it may have unwanted (in)direct commercial consequences for the fishermen. A better knowledge of the (side-)effects of electric pulses on marine organisms is therefore warranted and also demanded by ICES (2009) before a general introduction of electrotrawls can be authorized.

Unfortunately, the knowledge of possible side-effects on marine animals is limited to a few, often explorative, reports examining the effect of electrotrawl specific electric pulse and gear settings. The explanatory strength of these data is restricted to the particular pulse and electrode settings used. This is a drawback since the use of commercial pulse settings is not standardized and varies between vessels as a consequence of further developments by different producers, personal preferences of fishermen and seasonal variations in conductivity and fish behaviour. Moreover, new developments may further broaden the range of electric pulse settings used. Hitherto, the general goal of this thesis was to assess the effects of electric pulses on adult marine organisms based on their behaviour during and after exposure, the presence of macroscopic and histological injuries and their 14 day survival. Therefore, the animals were exposed to a variety of electric pulses to determine the safe upper limit that did not cause unacceptable side-effects. The pulse range tested also includes both electrotrawl pulses that are already used commercially, but it also explores the range of pulse (settings) that can be used in future applications. Since it is impossible to include all marine species, a limited number of species ('model-species') were used representing invertebrates (polychaetes, shrimp) and vertebrates (flatfish, roundfish). Doing this we should be able to demonstrate differences in reactions and sensitivity to lesions induced by electric pulses in this model-species. Subsequently, if adverse effects of the pulses of commercial electrotrawls were observed, additional experiments were performed using the specific pulse and electrode settings of these electrotrawls. Finally, this research was fed backed to the field, by investigating a new innovative application with the potential to further reduce bycatches in beam and electro trawl fisheries.

# Specific aims

Part I: Assessing the safe range of electric pulses for **invertebrates**.

- Are brown shrimp and ragworm negatively affected by the electric pulses used by electrotrawls in a standardized set-up?
- Is varying and increasing the pulse parameters a possible threat for brown shrimp and ragworm?
- Is the impact of repetitive exposure to electrotrawl larger than that of conventional beam trawls?

Part II: Determining the safe range of pulse settings for **flatfish**.

- Is sole negatively affected by the electric pulses used by electrotrawls in a standardized set-up?
- Is varying and increasing the pulse parameters in this set-up, be a possible threat for sole?

Part III: Investigating the sensitivity of **roundfish**

- Do cod show the same reaction and side-effects when exposed in a standardized set-up?
- Can we decrease and/or eliminate the occurrence injuries by changing the pulse parameter settings? What is the decisive pulse parameter?

- Which factors are responsible for the observed variability in cod's sensitivity for electric pulses?
- Do other roundfish, such as seabass, demonstrate a similar sensitivity for spinal injuries as cod?

Part IV: electrofishing's potential for **further innovation** and increased selectivity

- Can the different net design of electrotrawls and/or the post-catch use of electric pulses further increase the selectivity and reduce the bycatches of (electro)trawls?

# Rationale of the experiments

## *Choice of experimental animals*

Since the pulse exposure studies had to supply as much information as possible, the lab animals included had be representative for the different species encountered in electrotrawls catches. Therefore, two invertebrate model species, one flatfish and one roundfish species were chosen based on the following three criteria: (i) it should play a key-role in the ecosystem, (ii) it should show a good survival in captivity and (iii) it should have a commercial value for the fishery. The first part of this PhD focusses on invertebrates and used brown shrimp and ragworm as model species for invertebrates. Both benthic invertebrates meet the above criteria and live in close association with the sea floor and are therefore very likely to be exposed to electric pulses during electrotrawling. The second part uses sole as model species to examine the effects on flatfish, as these species have not been investigated yet despite being electrotrawls major target. Third, Atlantic cod was included as roundfish, as this fish is a top predator of the epifaunal and is reported to be most sensitive for injuries when exposed to electric pulses (van Marlen *et al.*, 2014; de Haan *et al.*, 2011). Additionally, it was decided during the experiments to perform also an identical exposure as with seabass to compare its sensitivity with that of cod and investigate variability amongst roundfish.

## Choice of the electrode set-up

The distribution of an electric field in the water can be homogenous or heterogenous. A *homogenous electric field* can be achieved by using plate shaped electrodes. This results in constant field strengths in the whole area between the two electrodes. When using this set-up, it is assumed that the electric field felt by the fish is unaffected by its position in the tank, as long as its orientation towards the electrodes is maintained. This eliminates many of the electric variables that are encountered in the field and allows the evaluation of a single parameter in a more standardized set-up. In contrary, wire-shaped electrodes used in commercial fishing practice generate a *heterogenous electric field*. This is characterized by very high field strength near the conductor that decreases exponentially towards the middle between 2 electrodes. As a consequence, (parts of the) fish close to the conductor will experience stronger electric stimulation compared to those further away from it and small changes in location and orientation result in changes in the amount of electricity passing the fish's body. Additionally, the diameter of the wire-shaped conductor will also strongly influence the electric field distribution. Nevertheless, when assessing the effect of a specific pulse gear, the use of the same wire-shaped electrodes mimics the field situation much better. This has the advantage that the obtained results can be extrapolated to the field more easily, but this set-up requires a good fixation of the fish at a certain location and an accurate description of electrode and pulse settings as well as the exact position of the fish.

## Choice of pulse settings

Electric pulses are defined by a set of parameters, i.e. frequency, pulse amplitude/field strength, pulse durations, pulse shape, pulse type and exposure time. To demonstrate an possible effect of each parameter, animals need to be exposed to a specific pulse parameter combination in which only one parameter is varied. Depending on the results, other approaches are required. This could include “worst-case” scenario’s in which animals are exposed to the most severe pulse parameters settings as can possible be present in commercial settings, eg. field strength of 200 V $m^{-1}$, frequency of 200 Hz, pulse duration of 1000 μs and an exposure time of 5 s.

### *Choice of the experimental design*

Examining the effect of different pulse parameters prefers a standardized design with minimum variability in field strengths. As a consequence, a homogenous set-up is recommended as it greatly simplifies experimental conditions and facilitates determination of cause and effect. Hitherto, plate shaped electrodes were used when examining the safe range and the effects of certain parameters (Chapter 3 & 5). The major drawback of this approach is that the obtained results cannot directly be extrapolated to normal electrofishing operations. Because side-effects in shrimp could not completely be ruled out, further experiments were done with the wire-shaped electrodes and pulse settings of commercial electrotrawls (Chapter 4). This was also done for cod and seabass (Chapter 6 & 7) to allow comparison with previous studies and extrapolation to the field.

## Outline of the thesis

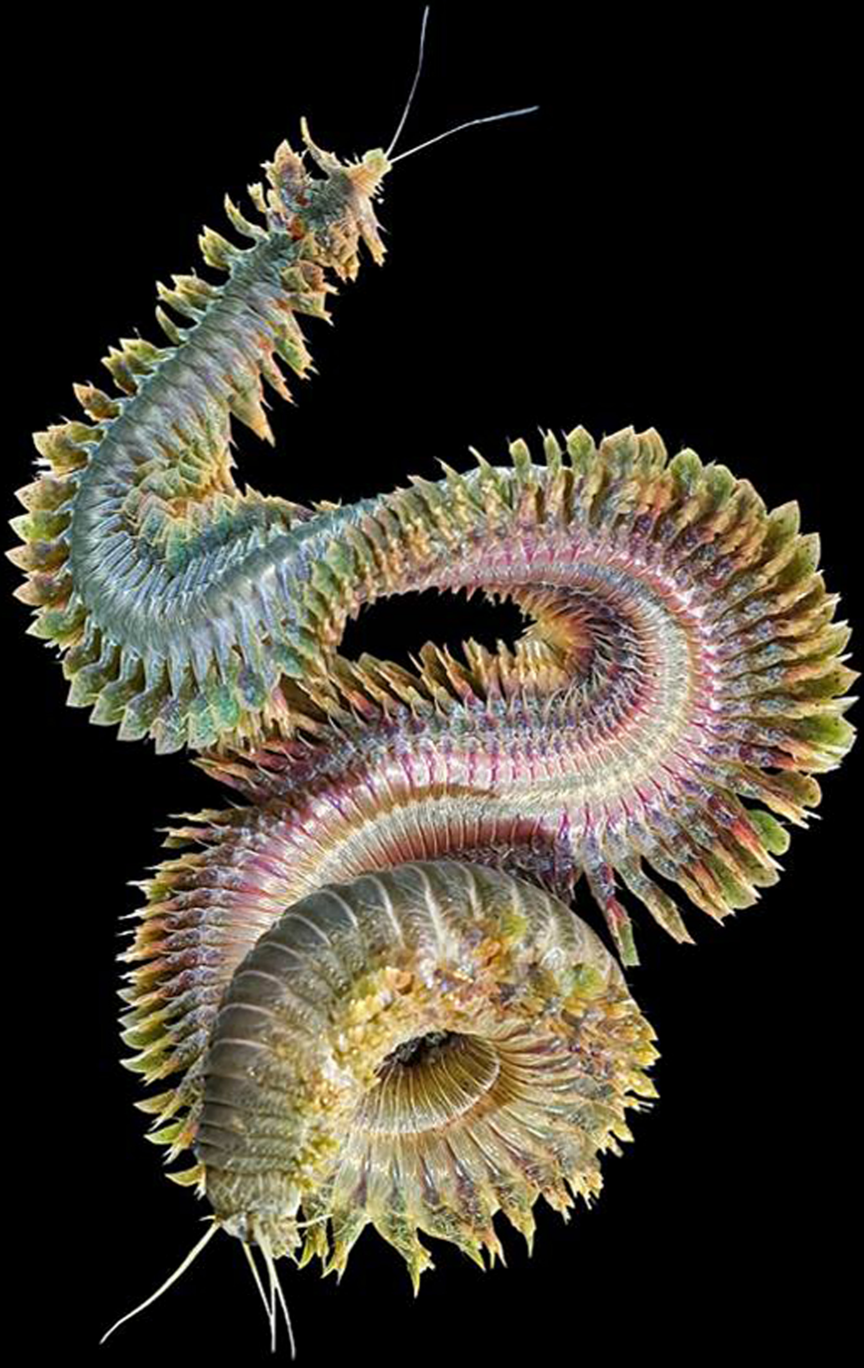
This thesis starts with the assessment of the impact of electric pulses on <u>invertebrates</u>. First, groups of **brown shrimp and ragworm** were exposed to the different pulses between plate electrodes. The aim is to determine how maximizing the different pulse parameters affects these species. At the same time, the effect of the pulses used by commercial electrotrawls were tested (**Chapter 3**). Second, brown shrimp were exposed repetitively to electric pulses between commercial wire-shaped electrodes as well as to mechanical stimulation of a tickler chain. Additional to the previous experiment, the effect on egg-carrying individuals and the percentage of moulting was included (**Chapter 4**).

The second part focused on sole as model species for flatfish. **Sole** were exposed individually between plate electrodes to 47 different electric pulses using both perpendicular and parallel orientation. Frequency, field strength, pulse duration and exposure time were varied from low to very high values and different pulse types and shapes were tested (**Chapter 5**).

Thereafter, the vulnerability of roundfish was tested in the third part. In a first study **cod** was exposed between plate electrodes (homogeneous set-up) and altered some pulse parameters to determine the decisive pulse parameters (**Chapter 5**). However, to more closely mimicking the field situation, cod was also exposed to electric pulses using wire-shaped electrodes (heterogeneous set-up). The goal of the subsequent experiments was to explain the large differences in sensitivity observed between different experiments with different cod, focusing on experimental set-up used and morphological differences between the cods studied (**Chapter 6**). Because cod is known to be very sensitive to electric pulses and difficult to obtain, additional experiments were performed with **seabass**. This species is indeed easier to obtain and could therefore serve as an alternative 'model-species' for roundfish. Additionally, linking morphological differences between both roundfish species to differences in effects could also provide useful indications of the decisive fish parameters (**Chapter 7**).

In the fourth and last experimental chapter, a potential new application of electric pulses was tested, aiming for further improved selectivity and reduction in bycatches. First, it was studied how the rectangular trawl design of electrotrawl may facilitate the use of a benthos release panels (BRP). These BRP are large meshed panels in the bottom of the net in front of the cod-end, through which bycaught trash, invertebrates and undersized fish can escape. Second, it was tested if the application of electric pulses in the trawl, after the animals had been caught, could further improve the selectivity. Therefore, an **electrified benthos release panel** (eBRP) was tested by adding electric pulse stimulation to the BRP, to prevent sole from escaping through this panel (**Chapter 8**).

The last part and chapter gives a **general synthesis** of the presented experiments as well as other research done on electrotrawls. It discusses on the strengths and weaknesses of the used experimental designs, it tries to assess the relevance of the obtained data for commercial fishing practice and to compare the total impact of electrotrawls with that of conventional beam trawls. It also elaborates on how electric pulses can create new opportunities and further increase the selectivity. Finally, it includes recommendations for further research (**Chapter 9**).



# CHAPTER 3

# DETERMINING THE SAFETY RANGE FOR TWO BENTHIC INVERTEBRATES: BROWN SHRIMP AND RAGWORM

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Adapted from:

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

# Abstract

Pulse trawling is currently the most promising alternative for conventional beam trawls targeting sole and shrimp, meeting both the fisherman'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 electric pulses on marine organisms. The organisms used in the present experiments were brown shrimp (*Crangon crangon* L.) and ragworm (*Alita virens* S.) as model species for crustaceans and polychaetes, respectively. These animals were exposed to a homogenously distributed electric field with varying values of the following parameters: frequency (5-200 Hz), electric 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 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, 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.

# 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 (Jones, 1992; Fonteyne *et al.,* 1998; Poos *et al.*, 2013). 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 by-catch (Lindeboom *et al.,* 1998; Bergman & van Santbrink, 2000; Jennings *et al.,* 2001). 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 the discard ban and further stated that unwanted by-catch 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 electric 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 500 µs 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 0.25-380 µs 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.* (2015). These electric pulses generated by electrodes affect the target species more selectively than beam trawling, thus reducing both by-catch and fishing effort (Soetaert *et al.*, 2015). Removing the tickler chains or reducing the number of bobbins addresses the main problems with beam trawling, i.e. seafloor 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 nr 850/98, article 31: non-conventional fishery techniques). But in 2009, Member States were granted a derogation by means of 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 electric 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 to 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 electric pulses on invertebrates. Studies evaluating the effects of electric pulses on invertebrates are limited and restricted to the pulse used to catch sole (i.e. 60-80 Hz versus 5 Hz for sole and shrimp, respectively). Smaal and Brummelhuis (2005) exposed 19 species of molluscs, echinoderms, crustaceans and polychaetes to electric 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 three 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 to the control groups, they observed a significant reduction in the survival rate of exposed 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 of 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 electric pulses used to catch sole, it is plausible that the effects of pulse beam trawling are far less invasive than the effects of conventional beam trawling.

However, a full assessment of the possible side-effects of electric pulses should go beyond merely testing the sole pulse. Indeed, all parameters inherent to electric 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.,* 2015). Moreover, besides merely assessing mortality and aberrant behaviour, microscopic examination of the exposed invertebrates undoubtedly adds value when investigating the effects of electric 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 electric parameters and their combinations on invertebrates using behavioural analyses and data retrieval on mortality, complemented by macroscopic and microscopic observations.

For this study, brown shrimp and ragworm were chosen as model species for the taxa crustaceans and polychaetes, respectively. Both benthic taxa live in close association with the sea floor and are therefore very likely to be exposed to electric 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 indirect economic value and play an important role in the food web. 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.

## Materials & Methods

### *Animals*

In total, 1730 brown shrimp (*Crangon crangon* L.) were included in this study. The shrimp were caught two days 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 (62 ± 4 mm) were captured off the Belgian coast using the research vessel Simon Stevin, equipped with a 4m 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 (66 ± 50 mm) 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; 188 ± 38 mm) were purchased from a commercial farm in the Netherlands (Topsy Baits, Wilhelminadorp, NL) and acclimatised in the experimental facilities for 1 week before starting the experiments.

### *Housing facilities*

The animals were randomly divided in different experimental groups of 30-60 (shrimp) or 23-50 (ragworm) individuals and housed in a series of 18 PVC aquaria (0.75 m L x 0.55 m W x 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; <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), while 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 two days 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 electric field with a constant electric field strength value between the electrodes.

**Table 3.1**: Overview of all the tested electric 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 |
| $S_e$ | 150 | 60 | 250 | 1.5 | PDC | e | 2 | 1 | ra |
| $S_q$ | 150 | 60 | 250 | 1.5 | PDC | q | 2 | 1 | ra |
| T,60 | 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: electric 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 with positive and negative part separated in time; s: square shaped pulse; e: exponentially shaped pulse; q: quarter sinus shaped pulse; sh: shrimp; ra: ragworm.

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 of the parameters were controlled by 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 x 0.4 x 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, 1972b). Brown shrimp were exposed to nine pulse parameter combinations in two experiments (Table 3.1). 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 electric field was not activated.

Prior to the exposure of shrimp, the cover of the tank was removed and the electrodes were gently inserted in the water. 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 means of a video camera (Sony ExmorR Handycam 12Mp) 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 3.2). All animals were monitored until 14 days 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.

**Table 3.2**: Scoring of reaction of brown shrimp and 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 immed. | minority squirms |
| | 2 | all burrowed after 1s | majority squirms |
| | 3 | all burrowed after 2s | 100% squirms, minority strongly |
| | 4 | all burrowed after 3s | 100% squirms, majority strongly |
| | 5 | some still jumping >3s | 100% strongly squirms |

## *Pulse parameters*

Five pulse parameters were used. The electric 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 3.1. 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 with a positive and negative part in each pulse (PAC), and a bipolar pulse with alternating a positive and a negative pulse (PBC), as illustrated in Figure 3.1. Additionally, also the duty cycle (dc, [%]) is mentioned, which combines the frequency and pulse duration giving the time proportion during which electric 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 electric pulses were applied, and the number of exposures. The range in which these parameters were varied is given in Table 3.1.

The nominal pulse settings were 60 Hz, 150 V $\text{m}^{-1}$, 250 µs with a square pulse shape. 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 day 0, 5, 8 and 11 to simulate possible repetitive exposures of animals in the field. As indicated in Table 3.1, ragworm was exposed four times during the first 10 days to a pulse in which all parameters were set to the maximum (R), while brown shrimp was exposed repeatedly to the pulses used in the shrimp fishery ($R_{cr}$) and the flatfish fishery ($R_{fl}$).

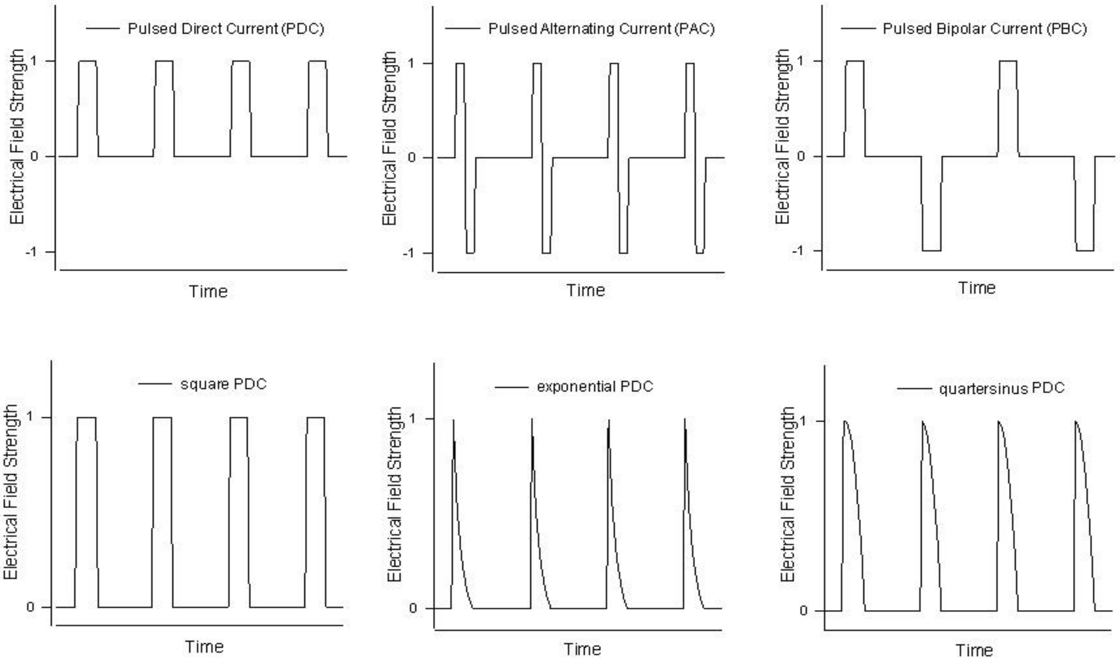


**Figure 3.1**: An illustration of the 3 pulse types and 3 pulse shapes used during the experiments.

## Gross & histological examination

Two weeks following exposure to the electric pulses, ten animals were sacrificed by means of 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 & Lightner (1988) and Hopwood (1996). Briefly, the carapax and tail of 10 individuals per group were separated and fixed in Davidson fixative (Bell & 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 (HE) 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 numerous hepatopancreatic tubuli); 3= abundant (majority of hepatopancreatic tubuli contain few aberrant nuclei); 4 = severe (majority to all of the hepatopancreatic tubuli contain numerous aberrant nuclei).

Ten ragworms per group were fixed in a 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 segment), oesophagus (7th + 8th segment) and intestine (11th + 12th segment) region. 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 (MMA) were encountered was recorded. In addition, the number of

MMA in each of the organs was counted blind per section and scored from 0 to 3 as follows: 0 (no MMA), 1 (1-2 MMA), 2 (3-5 MMA) or 3 (>5 MMA).

### *Statistics*

For shrimp, the percentage mortality rate after 7 and 14 days, the average size difference between dead and surviving individuals and the mean virus load of IBV was obtained per aquarium and analysed using a mixed model with pulse exposure as categorical fixed effects factor and replicate as 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 days was based on the exact logistic regression model. The number of animals affected with MMA 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*

The results are summarized in Table 3.3. In general, 95-100% of the shrimp reacted with a tail flip when exposed to electric 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 (0.2m). A frequency of 60 or 200Hz and higher resulted in one powerful contraction that made them jump 0.1-0.15m 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 to 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. All control animals remained buried in the sand without tail flipping behaviour.

**Table 3.3**: Results of behaviour scoring during and after exposure, the survival rate at 7 and 14 days after exposure and the severity scoring for intranuclear baciliform viruses (IBV) after exposure of brown shrimp to different electric pulses.

| **Study *** | **Pulse ID** | **Median behaviour Score (± s.d.)** | | **Mean percentage survival (± s.d.)** | | **Mean IBV score (± s.d.)** |
|---|---|---|---|---|---|---|
| | | **during** | **after** | **7 days** | **14 days** | |
| 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) |
| | Rcr | 5 (±0) | 3 (±1) | 93.3 (±4.1) | 66.1 (±9.5) | 1.5 (±1.0) |
| | Rfl | 5 (±0) | 3 (±1) | 95.6 (±0.8) | 75.6 (±7.5) | 1.0 (±1.1) |

* the first study was repeated at three time points; the triplicates of the second study were performed simultaneously; s.d.: standard deviation.

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 of 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 mortality rate was observed in all groups (Table 3.3). None of the tested parameter combinations resulted in a significantly higher 7-day or 14-day 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 to the control group ($P_{adj}$=0.0166), the PAC60 group ($P_{adj}$=0.0255) and the F200 group ($P_{adj}$=0.0049). When compared to Db60, the difference was not significant ($P_{adj}$=0.1523). No significant increase compared to 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 coast line for the 2nd experiment (67% infected, average score = 1.07±1.00). This may be due to environmental stressors such as pollution (Stentiford & Feist, 2005), with pollutants generally reaching concentrations in coastal water such as Ostend's harbour mouth, which was located less than 500 m from the beach where the shrimp were foot trawled.

## *Ragworm*

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, while 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.

The results are summarized in Table 3.4. During the 14-day follow-up period, the mortality varied between 0% and 4%. None of the tested parameter combinations resulted in significantly higher 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 3.4**: Results of survival, presence and scoring of melanomacrophage aggregates 14 days after exposure of ragworm to different electric pulses

| **Pulse ID** | **# Animals** | **14d Survival** | **Melanomacrophage Aggregate** | |
|---|---|---|---|---|
| | | | **Presence (% animals)** | **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) |
| Se,60 | 23 | 100.0% | 10% | 0.4 (±1.26) |
| Sq,60 | 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) |

Legend: Experiments indicated with '*' were done with ragworms that were not fed during 1 month prior to exposure.

# Discussion

## *Experimental setup*

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 electric field with constant electric 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 setting 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 electric potential experienced by the animal. Furthermore, the electric pulses in the experiments were raised 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 to the commercial flatfish pulse trawlers, resulting in duty cycles up to 6% whereas the maximum duty cycle in the field is 2.2% (Soetaert *et al.*, 2015a). This signifies that the electric 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 5Hz 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 raised 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 60Hz and higher, the contractions of the muscle induced by the electric 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 around 20 Hz (Snyder, 2003a). 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 upward jump seen at higher frequencies followed by escape behaviour might offer an alternative way to catch brown shrimp using electric pulses. The current electrofishing on sole is based on pulses with frequencies of 40 to 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 possibly 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 & Brummelhuis, 2005; van Marlen *et al.*, 2009). Only common prawns stayed immobilized until 1 minute after exposure (Smaal & Brummelhuis, 2005; van Marlen *et al.*, 2009). This cramp reaction is not observed in other invertebrates. Echinoderms (starfish, sea urchin, brittle star) show no reaction, while razor clams can even use their foot and siphon, often exhibiting strong enough reactions to even propel them away from the pulse

(Smaal & Brummelhuis, 2005; van Marlen *et al.*, 2009). Molluscs (cockle, prickly cockle, whelk, netted dog whelk, 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 & 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 & 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 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 group, which results in higher field strengths inside the body compared to the exposure of a single animal, as described by D'Agaro *et al.* (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 sub-lethal 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 electric pulses were either absent or had healed 14 days following exposure for brown shrimp as well as 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 & 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 two-week monitoring period in the lab, 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 days. 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 to previous exposure experiments on invertebrates, our results confirm those obtained by Smaal & 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, while razor clam showed

6% higher survival compared to the control animals. Similarly, 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, while razor clam and ragworm had a 7% lower survival compared to non-exposed animals. When all groups were clustered, a 3-5% lower survival rate was found for ragworm and European green crab, respectively, after exposure to the flatfish pulse. No correlation between higher electric 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 insufficient 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 fundamental 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 days 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 as well as day 14 post-exposure.

# Conclusion

Brown shrimp and ragworm were used as model species to investigate the short-term effect of electric 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 days after exposure, affirming 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 electric field strengths, warranting further research.

# CHAPTER 4

# IMPACT OF REPETITIVE EXPOSURE TO ELECTRIC PULSES ON BROWN SHRIMP

Adapted from:

Soetaert, M., Verschueren, B., Chiers, K. Duchateau, L., Polet, H. & Decostere, A. 2015. Impact of repetitive electric exposures on brown shrimp (*Crangon crangon* L.). Submitted to Journal of Marine and Coastal Fisheries, November 2015.

# Abstract

Pulse trawling is currently the best available alternative for beam trawling in the brown shrimp and sole fishery. To evaluate the effect of repetitive exposure to electric fields, brown shrimp was exposed 20 times in 4 days using commercial electrodes and pulse settings to catch shrimp (shrimp startle pulse) or sole (sole cramp pulse) and monitored for 14 days post first exposure. The survival, egg loss, moulting and the degree of intranuclear bacilliform virus (IBV) infection were evaluated and compared to stressed but non-electric-exposed and non-stressed non-exposed shrimp as well as to shrimp exposed to mechanical stimuli. The lowest survival at 14 days post first exposure was observed for the sole cramp pulse treatment (57.3%), which was significantly lower than that of the non-electric-exposed control group with the highest survival (70.3%). The lowest percentage of moults was observed for the repetitive mechanical stimulation treatment (14.0%) which was significantly lower than that of the non-electric-exposed control group displaying the highest percentage of moults (21.7%). Additionally, the mechanically stimulated shrimp that died during the experiment had a significant larger size compared to the surviving individuals. Finally, no effect of electric stimulation on the IBV infection was found.

# Introduction

Pulse trawling is currently the best alternative available for conventional beam trawling. In these electrotrawls, the mechanical stimulation by tickler chains, chain mats or bobbins is (partly) replaced by electric stimulation through wire-shaped electrodes. These electrodes consist of a series of isolated and conductive parts and are connected to the beam trawl or its alternative (sumwing, seewing, multiwing, …) and towed over the seabed, followed by a footrope or straight bobbin rope with a reduced number of bobbins (Soetaert *et al.*, 2015). In a previous study with shrimp (*Crangon crangon* L.), exposure to a single electric pulse with varying pulse parameters, generated by plate-shaped electrodes, did not result in a higher mortality nor in lesions (Soetaert *et al.* 2014). However, shrimp exposed to the highest electric field strength showed an increase in severity of intranuclear bacilliform virus (IBV) infection in the hepatopancreas. In addition, following a four-fold exposure to two commercially used electric pulses to catch shrimp or sole during a 10-day period, no discernible negative effects were found (Soetaert *et al.* 2014). This rectifies the promising character of this alternative fishing technique. However, no data are available on the impact of exposure to commercial wire-shaped electrodes generating a heterogenous electric field, nor the effect of multiple exposures experienced by shrimp in a short period of time, as may occur in fishing practice.

The purpose of the present study was to evaluate the effect of repeated exposures on shrimp to the startle and cramp pulse used in the field for catching shrimp and sole, respectively. Survival, moulting, macroscopic and microscopic lesions were adopted as parameters. Moreover, gravid female shrimp were included in the study, enabling the impact assessment on egg carriage.

# Materials & Methods

## Animals & housing facilities

In total, 1079 brown shrimp with an exoskeleton length of 64.4 ± 6.5 mm were included. The shrimp were caught along the Belgian coast with a commercial 4 m shrimp beam trawl and transported to the housing facilities within 3 h. They were allowed to acclimatise during 5 days and housed and fed as described by Soetaert *et al.* (2014). Shrimp were randomly divided over 18 experimental tanks of 58-60 animals (consisting of 38-41 shrimp with eggs and 19-20 shrimp without eggs). The water parameters were as follows: 12 °C temperature; 35‰ salinity; 4.29 S $m^{-1}$ conductivity; 8 pH; 6 °KH; <25 mg $L^{-1}$ nitrate; <0.2 mg $L^{-1}$ nitrite; <0.1 mg $L^{-1}$ ammonia.

## Electric set-up used

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. Wire-shaped electrodes, such as are used in the commercial fishery were adopted as described in Soetaert *et al.* (2015). For the shrimp startle pulse, two electrodes of 0.5 m length were placed on a mutual distance of 0.6 m (Figure 4.1a). A 5 Hz pulsed direct current with a pulse duration of 500 µs was applied. For the sole cramp pulse, two 0.15 m conductors were placed on a mutual distance of 0.42 m in the centre of the tank (Figure 4.1b). An 80 Hz pulsed bipolar current with a pulse duration of 250 µs was applied. All electrodes were mounted in a 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 set-ups, 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 optimally copy the field situation.

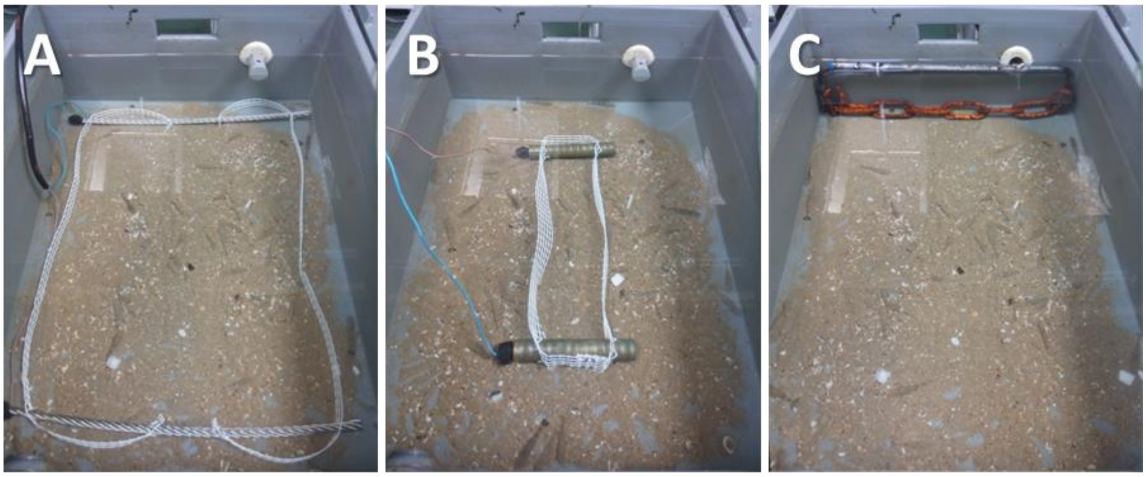


**Figure 4.1:** The experimental set-up for the shrimp startle pulse (A), sole cramp pulse (B) and mechanical chain stimulation (C). For the electric stimulation of shrimp (A&B), power leads are entering the tank from the left and connected to the end of the electrodes, which are fixed at a mutual distance and at 1 cm above the bottom of the tank by means of white PVC nettings 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 $s^{-1}$.

## Experimental design

Five set ups were included: no stressor ('CTRL'; 243 animals over four replicates) or repeated exposure to the shrimp pulse ('SHRI'; 241 animals over four replicates), to the sole pulse ('SOLE'; 238 animals over four replicates), to electrodes without a pulse ('ELEC'; 179 animals over three replicates) or to a mechanical stimulus ('MECH'; 178 animals over three replicates). Immediately prior to the first exposure, 30 shrimp (15 with and 15 without eggs) in total 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 (IBV) infection in the hepatopancreas was examined and an average score was given as previously described (Soetaert *et al.,* 2014).

Shrimp of the SHRI and SOLE groups were exposed to electric pulses as described above. Prior to each exposure, 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. After the shrimp had resettled in the sediment, the electrodes were removed gently and the tank was covered again. Shrimp of the ELEC-group were treated in the same way as the SHRI and SOLE-group, but no electric stimulus was applied. Shrimp of the MECH group were mechanically stimulated using a chain mounted on a U-shaped grip that was pulled at approximately 1 m $s^{-1}$ through the tank (Figure 4.1c). All stimuli were applied five times a day with 90 min intervals during four successive days, where after the shrimp were kept further for another 10 days. Dead individuals and shrimp moults were removed daily. The number and exoskeleton size of dead shrimp were recorded separately for shrimp with and without eggs. Fourteen days post first exposure (DPFE), the number of surviving shrimp with and without eggs was determined for each tank. Three shrimp with and three without eggs from each replicate were randomly selected, sacrificed, measured and processed for histological examination as described above. The percentage of moults was defined as the ratio between the number of moults and the number of animals initially stocked in the tanks, x 100. The percentage of shrimp with loss of eggs at 14 DPFE was calculated by dividing the sum of dead shrimp with eggs and surviving shrimp with eggs by the total number of shrimp with eggs at the start of the experiments.

### *Statistics*

The statistical analysis investigated differences between treatments in 7 and 14 DPFE survival, 14 DPFE egg loss and 14 DPFE percentage moults using the generalised mixed model with binomially distributed error term and replication as a random effect. Each time, nine pairwise comparisons were made (CTRL vs MECH, SHRI, SOLE; ELEC vs MECH, SHRI, SOLE, MECH vs SHRI, SOLE and SHRI vs SOLE), setting the comparison wise level for significance at 0.05/9= 0.0056. The difference between the size of dead and survived shrimps at 14 DPFE is based on the fixed effects model and is done separately for each treatment. The size of all shrimp that had died is also compared between the different treatments using the fixed effects model. The effect of treatment on the IBV-score was done based on the Kruskall Wallis test and the correlation with 7 and 14 DPFE mortality was determined using Kendall's correlation coefficients.

## Results

Shrimp exposed to the shrimp pulse (SHRI group) showed startle behaviour, while shrimp subjected to the sole pulse (SOLE group) displayed a cramp reaction, both followed by an escape response as described by Soetaert *et al.* (2014). However, the shrimp of the SOLE group located in the corners of the tank demonstrated less intensive cramp behaviour with attenuated tail flipping as compared to animals situated in the centre. Animals exposed to the mechanical stimulus (MECH group) either immediately reburied or showed a short escape reaction. Shrimp from the CTRL or ELEC groups did not display a tail flipping reaction and all animals remained buried in the sand, except when accidently touched.

**Table 4.1:** The mean percentage (± s.e.) of shrimp that survived at 7 and 14 DPFE and had molted or lost eggs after 14 DPFE. Significant differences are indicated in superscript.

| Group* | 7 DPFE survival | 14 DPFE survival | 14 DPFE moults | 14 DPFE egg loss |
|---|---|---|---|---|
| CTRL | 88.9 ± 4.1 | 66.3 ± 4.7 | 18.1 ± 2.0 | 30.6 ± 5.4 |
| ELEC | 88.8 ± 1.5 | 70.3 ± 4.2$^{(1)}$ | 21.7 ± 1.4$^{(2)}$ | 29.3 ± 5.4 |
| MECH | 82.0 ± 5.0 | 60.1 ± 5.0 | 14.0 ± 2.2$^{(2)}$ | 25.6 ± 9.0 |
| SHRI | 85.9 ± 2.7 | 65.1 ± 2.6 | 18.7 ± 3.3 | 31.7 ± 1.9 |
| SOLE | 82.9 ± 1.4 | 57.3 ± 3.7$^{(1)}$ | 18.3 ± 2.3 | 31.2 ± 4.5 |

In all groups, the food response declined gradually over time, with food left-overs from 8 DPFE onwards. The percentages of animals that survived, moulted, and/or lost eggs are given in Table 4.1. A significantly lower 14 DPFE survival for the SOLE group when compared to the ELEC group (P=0.0034, αadj=0.0056) was found, and a significantly lower percentage moults for the MECH group compared to the ELEC group (P<0.0001, αadj=0.0056). When the size of shrimp that died in different groups (Table 4.2) was compared, no differences were found, but comparing the size of surviving and death shrimp within each group, it showed that the surviving shrimp of the MECH group were significantly smaller than those that had died (P=0.0175, α=0.05).

**Table 4.2:** The mean values (± s.e.) of the size of the shrimp that died during the experiment, those that survived the experiments and the severity scoring for intranuclear baciliform viruses (IBV) before and 14 DPFE for each group.

| Group* | Size (mm) | | IBV-score |
|---|---|---|---|
| | Dead 14 DPFE | Survived 14 DPFE | Survived 14 DPFE |
| CTRL | 65.0 ± 0.8 | 64.1 ± 0.5 | 1.88 ± 0.19 |
| ELEC | 66.1 ± 0.9 | 64.1 ± 0.6 | 1.11 ± 0.29 |
| MECH | 65.2 ± 0.8 | 62.8 ± 0.6 | 1.33 ± 0.26 |
| SHRI | 64.6 ± 0.7 | 63.9 ± 0.5 | 1.17 ± 0.21 |
| SOLE | 66.1 ± 0.7 | 64.4 ± 0.6 | 1.21 ± 0.20 |

* CTRL = no stressor, ELEC = electrodes without pulse, MECH = mechanical stimulation by means of a towed chain, SHRI = commercial set-up to catch shrimp, SOLE = commercial set-up to catch sole.

Histological examination did not reveal acute/subacute lesions. However, in 5% of the examined individuals an intramuscular nematode was present. No eggs were found in the stomach of the shrimp. The average score for the IBV prior to exposure was 1.54 ± 0.22. This score increased during the 14 d experiment for the CTRL group, but decreased for all other treatments as presented in Table 4.2. Statistical analysis did not demonstrate significant differences between the IBV scores of the different groups. In addition, no correlation was found between mortality and the IBV-score.

# Discussion

The present experimental set-up was designed to simulate commercial electrotrawling on sole as accurately as possible, by using commercial wire-shaped electrodes, generating a heterogenous electric field. This set-up resulted in a higher variation in possible electric doses experienced by the animals, which is in contrast to a previous study with plate-shaped electrodes (Soetaert *et al.*, 2014). Indeed, the intensity of the experienced electric field decreases exponentially with the distance between the shrimp and the electrodes, which was illustrated by the less pronounced cramp reaction of individuals situated in the corners of the tanks. This observation confirms that no effective stimulation outside the trawl path is to be expected (De Haan *et al.*, 2011) and that The electric experimental set-ups in the present study resulted in increased electric field strengths compared to what is encountered in practice. In the SHRI set-up this resulted from the reduced distance between the electrodes, which was limited to 0.6 m due to tank size limitations compared to 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 the electrotrawls (Soetaert *et al.*, 2015). Additionally, the sequence of exposures, 20 times in one fishing week of 4 days, is most likely much faster compared to 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 set-up represents a “worst-case” scenario of exposures to electrotrawl pulses.

The effect on survival differed depending on the pulses used. The lowest survival at 14 days post first exposure was observed for the SOLE group exposed to 80 Hz pulses, which was significantly lower than the ELEC group with the highest survival (70.3%). No significant differences were found with the 5 Hz pulses used to startle shrimp (SHRI), suggesting no negative effects of this stimulus on shrimp. The difference may be explained by the electric load, as the duty cycle, which is the portion of time that electric current is effectively running, was 8 times longer for the SOLE group compared to the SHRI group. This effect was not observed in a previous study using the same pulse in a homogenous set-up with more intense but less frequent exposures (Soetaert *et al.*, 2014). Therefore, it remains to be elucidated whether this represents a consistent finding.

The percentage of moults and egg loss was monitored in the present experiments. This study hence is the first in employing these parameters to discern possible sub-lethal effects of repeated exposures and to investigate whether electrotrawling could interfere with reproduction. No significant effect of electric stimulation on egg loss or the percentage of moults was demonstrated. However, mechanical stimulation resulted in the lowest percentage of moults, which was significantly lower than the ELEC group. Besides, the MECH shrimp that died during the experiment had a significant larger size compared to the surviving individuals, which was not observed in other treatments. We hypothesize that this is related to the occurrence of physical injuries induced by the impact of the chain, either during passage or when arriving at the end of the tank, as larger shrimp have a higher probability to be impacted or crushed. Indeed, physical injuries have been shown to affect the survival adversely (Bergmann *et al.*, 2001; Depestele *et al.*, 2014), which may be elicited by damage of their fragile externalities (Kaiser and Spencer, 1995). In addition, this also demands extra energy with a subsequent decrease in growth and moult increments (Bennett, 1973), which would explain the difference in the percentage of moults encountered.

In a previous study, an increased IBV infection was demonstrated in the haepatopancreas of shrimp exposed to field strengths of 200 V $m^{-1}$ in a homogenous set-up, indicating a possible indirect effect of electric pulses (Soetaert *et al.*, 2014). In the present study, despite the repetitive exposures, such an increase was not observed. In contrast, the highest IBV infection was found in the non-stressed control group. We may speculate that shrimp with the highest IBV load died and since the IBV score was determined on surviving animals, this resulted in a lower mean. However, the lack of correlation between the severity of IBV-infection and mortality seems to reject this hypothesis. Another reason may be that in the heterogenous set-up as employed in the present study, field strengths of 200V $m^{-1}$ 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 shrimp and only during a very short time interval, as they immediately jumped out of this range. Therefore, the number of shrimp exposed to sufficiently strong and long pulses may be too low in the commercial electrotrawl set-up to obtain higher IBV-infection observations.

In conclusion, no negative side-effects were demonstrated in adult shrimp that were repetitively exposed to the commercial shrimp startle pulse. In contrast, shrimp exposed to the stronger cramp pulse to catch sole had the lowest survival, which was significantly different from that of the shrimp exposed to electrodes without electric pulses, displaying the highest survival. However, no differences in mortality were noted with shrimp that were not stressed or those exposed to the shrimp startle pulse or to mechanical stimulation. The repetitive mechanical stimulation of adult shrimp resulted a decreased percentage of moults and in size-specific mortality with larger shrimp showing a reduced survival. The latter findings urge us to speculate on the actual significance of the encountered negative impact of repetitive exposures to the sole pulse in shrimp. Indeed, any impairing effects of the sole pulse should be balanced against the harmful impact of the conventional trawls.

# CHAPTER 5

## SIDE-EFFECTS OF ELECTROTRAWLING: EXPLORING THE SAFE OPERATING SPACE FOR DOVER SOLE AND ATLANTIC COD

Adapted from:

Soetaert, M., Decostere, A., Verschueren, B., Saunders, J., Van Caelenberge, A., Puvanendran, V., Mortensen, A., Duchateau, L., Polet, H. & Chiers, K. (2015) Side-effects of electrotrawling: exploring the safe operating space for dover sole (*Solea solea* L.) and Atlantic cod (*Gadus morhua* L.).
Accepted in Fisheries Research.

# Abstract

Electrotrawling is currently the most promising alternative for conventional beam trawls targeting sole and shrimp, meeting both the fisherman's aspirations and the need for more environmentally friendly fishing techniques. Before electrotrawling can be further developed and implemented on a wider scale, more information is needed about the effects of electric pulses on marine organisms. Adult Dover sole (*Solea solea* L.) and Atlantic cod (*Gadus morhua* L.) were used in the present study as model species for flatfish and roundfish, respectively. These animals were exposed to homogenously distributed electric fields with varying values of the following parameters: frequency (5-200 Hz), electric field strength (100-200 V $m^{-1}$), pulse polarity, pulse shape, pulse duration (0.25-1 ms) and exposure time (1-5 s). The goal was to determine the range of pulse parameters which can be regarded as safe and thereby also to evaluate the effect of the pulses already being used in commercial electrotrawls. Fish behaviour during and shortly after exposure, 14-d post exposure mortality rates, as well as gross and histological examination was used to evaluate possible effects. During exposure, both species showed an escape response below a frequency of 20 Hz and a cramp reaction above 40 Hz. These reactions were immediately followed by post-exposure escape behaviour and at high electric loads cod showed tonic-clonic epileptiform seizures. No mortality was observed and histological examination did not reveal any abnormalities, except for one cod showing a spinal injury. These data reveal the absence of irreversible lesions in sole as a direct consequence of exposure to electric pulses administered in the laboratory, while in cod, more research is needed to assess cod's vulnerability for spinal injuries when exposed to the cramp pulses.

# Introduction

In traditional beam trawl fisheries, tickler chains, chain matrices or bobbin ropes are used to startle and catch flatfish or shrimp. These fishing gears are usually heavy and have a high drag, resulting in the well-known disadvantages for both the fishermen as the ecosystem including high fuel consumption, high by-catches and seabed disturbance (Jones, 1992; Fonteyne *et al.,* 1998; Poos *et al.*, 2013). This mixed fishery targets several species with highly variable minimum landing sizes, resulting in poor selectivity and thus high by-catches and discards (Lindeboom *et al.,* 1998; Bergman & van Santbrink, 2000; Jennings *et al.,* 2001). 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 by-catch should herein be reduced (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 matrices or bobbins is (partly) replaced by electric stimulation. The electric pulses generated by electrodes affect the target species more selectively, thus reducing both by-catch and fuel consumption by 50% and more (Rasenberg *et al.*, 2013; van Marlen *et al.*, 2014). The use of electricity to catch marine organisms was prohibited by the European Commission in 1988 (EC nr 850/98, article 31: non-conventional fishery techniques). Nevertheless, in 2009, EC member states were granted a derogation of 5% of the fleet to use the pulse trawls in the Southern North Sea, which was extended to 10% of the fleet in early 2014. At this moment, 91 vessels have already adopted this technique commercially, of which 1, 3, 10 and 77 have a Belgian, UK, German and Dutch license, respectively. Although most vessels differ in rigging and weight of fishing gear, the electric parameters are similar and can roughly be 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 Dover sole (*Solea solea* L.), by using a bipolar cramp pulse around 80 Hz to increase the catching efficiency. A minority of vessels target brown shrimp (*Crangon crangon* L.) by outfitting their boat with electrotrawls that produce a unipolar startle pulse of 5 Hz. However, before a general exemption on this fishery can be implemented, several concerns about negative effects of electric pulses on survival, behaviour and reproduction of target and non-target species need to be addressed (ICES recommendations, 2009).

In this respect, the purpose of this study was to fully assess the possible side-effects of electric pulses on adult Dover sole and Atlantic cod (*Gadus morhua L.*), as model species for flatfish and roundfish, respectively. Dover sole was chosen because it is the main target species of electrotrawls and data on the potential impact of electric pulses on this important commercial species are lacking. Atlantic cod is known to be the most sensitive species encountered in the catches of pulse trawls, which was illustrated in a catch comparison that revealed spinal injuries in 4 out of 45 cod caught (van Marlen *et al.*, 2014). This was confirmed in laboratory experiments showing that 50-70% of adult cod was harmed when they were exposed near the electrodes (40-100 V $m^{-1}$), while no injuries were observed when they were exposed at more than 0.2 m away from the electrodes (De Haan *et al.*, 2009a & 2011). In contrast, juvenile cod exposed to much higher field strengths of 250-300 V $m^{-1}$ did not reveal injuries (De Haan *et al.,* 2011).

The present study tested a broad range of electric parameters and their combinations, going beyond the above listed previous research on cod that merely included the sole pulse and solely adopted mortality and morbidity as criteria for impact assessment. Indeed, when a full safety profile of electric pulses is envisaged, all electric parameters should be included, varied and combined. Because a standardized design with minimum variability in field strengths is preferred when determining the effect of different pulse parameters, a homogenous electric field has been recommended (Soetaert *et al.*, 2014). When using this set-up, it is assumed that the electric field felt by the fish is unaffected by its position in the tank, as long as its orientation towards the electrodes is maintained. In addition, the present study investigated the occurrence of spinal injuries and associated haemorrhages are warranting the need for radiological and histological examination in safety assessment studies (Snyder, 2003a). Therefore, X-ray analysis and histological examination of the internal organs to discover sub-lethal effects were performed.

## Materials & Methods

### *Animals & housing facilities*

A total of 154 wild Dover sole (0.277 ± 0.028 m; 162 ± 59 g) and 14 wild (0.405 ± 0.038 m; 546 ± 133 g) and 46 farmed (0.704 ± 0.036 m; 4166 ± 723 g) Atlantic cod was included in this study. All wild fish were caught in the North Sea two months before the experiments were carried out. For sole, the Research Vessel (RV) Simon Stevin using a 4 m shrimp beam trawl or the RV Belgica using an 8 m beam trawl with chain matrix was used. Only short (± 20 min) fishing hauls were carried out in order to reduce stress and injury caused by the fishing process. The wild cod were caught with hook and line around windmills on the Blighbank (North Sea). Animals in good condition were transported in survival tanks continuously supplied with seawater to the ILVO housing facilities in Ostend, Belgium. Upon arrival, sole were housed in groups of three to four individuals in PVC aquaria of 0.7 m (L) x 0.55 m (W) x 0.30 m (H) with grey-coloured walls and a water level of 0.2 m, provided with aeration and a cover with limited light penetration to mimic natural conditions. The wild cod were kept in a tank of 2.75 m (L) x 1.00 m (W) x 1.20 m (H) with a black background and a water height of 0.9 m and a 12 h/12 h light/dark regime. Both fish species were housed in a recirculation system supplied with natural seawater and connected to a common matured and fully functional biological filter. The water quality was monitored regularly and kept at a constant level (15 °C; 35 ‰ salinity; 8 pH; 6° KH; <25 mg L V$^{-1}$ nitrate, <0.2 mg L$^{-1}$ nitrite, <0.1 mg L$^{-1}$ ammonia). None of the tanks were provided with substrate on the bottom. The animals were fed two to three times a week with mussels, shrimp or pieces of whiting or plaice (cod) or mussels and ragworm (sole). The farmed cod were purchased from the 4 year old brood stock of the Norwegian Cod Breeding Centre of NOFIMA (Tromsø, Norway). These PIT-tagged animals were kept in sea cages from July till January and during the spawning season transported to large circular tanks of 23 $m^3$, constantly supplied with natural seawater in a flow through system. The water temperatures ranged between 3.1 and 4.9 °C and the salinity was 34 ‰. The farmed cod were fed dry pelleted feed, daily (Vitalis Cal, Skretting, Spain). The experiments were approved by the Belgian (ID 2011/170) and Norwegian ethical committee (ID 5183).

## Pulse parameters

In total, six different electric pulse parameters were used: the electric field strength (E, [V m-1]), the frequency (F, [Hz]), the pulse duration (D, [μs]), the pulse shape (S), the pulse type (T) and the exposure time (L, [s]). Three pulse type were used: pulsed direct current (PDC), pulsed alternating current (PAC) and pulsed bipolar current (PBC). The pulse types and shapes used are illustrated in Soetaert *et al.* (2014). Finally, the duty cycle (dc, [%]) is also mentioned, which combines the frequency and pulse duration. The pulses to which sole and cod were exposed are listed in Table. 5.1 and Table 5.2, respectively.

**Table 5.1:** Overview of the 47 parameter combinations, to each of which 'n' sole were exposed. Each sole was exposed to one pulse ID, either in perpendicular or in parallel orientation toward the plate electrodes. The nominal pulse was a square PDC with a pulse duration of 250 µs and an exposure length of 2 s. The nominal field strength was 150 V m$^{-1}$ and 200 V m$^{-1}$ for an orthogonal and parallel orientation, respectively, unless indicated differently. Each pulse parameter was varied and tested at different frequencies, while keeping all other pulse settings constant at the nominal settings.

| **Parameter values** | | **Frequency (Hz)** | **Pulse ID's** | **n** |
|---|---|---|---|---|
| ***Sole (perpendicular)*** | | | | |
| Frequency * | | 5, 20, 40, 60, 80,<br>100, 120, 140,<br>160, 180, 200 | F5, F20, F40, F60, F80,<br>F100, F120, F140,<br>F160, F180, F200 | 2 |
| Pulse duration | 100 µs | 5, 60 | Da5, Da60 | 3 |
| | 1000 µs | 5, 60, 100 | Db5, Db60, Db100 | 3 |
| Field strength | 150 V m$^{-1}$ | 5, 60, 200 | Ea5, Ea60, Ea200 | 3 |
| | 200 V m$^{-1}$ | 5, 60, 200 | Eb5, Eb60, Eb200 | 3 |
| Pulse shape | quartersinus | 5, 60 | Sq5, Sq60 | 3 |
| | exponential | 5, 60 | Se5, Se60 | 3 |
| | sinusoidal | 5, 60 | Ss5, Ss60 | 3 |
| Pulse type | PAC | 5, 60, 150 | Ta5, Ta60, Ta150 | 3 |
| | PBC | 5, 60, 150 | Tb5, Tb60, Tb150 | 3 |
| Exp. length | 5 s | 5, 60, 150 | L5, L60, L150 | 3 |
| ***Sole (parallel)*** | | | | |
| Frequency | | 100, 200 | F100, F200 | 3 |
| | | 5, 60, 150 | F5, F60, F150 | 5 |
| Pulse duration | 1000 µs | 60, 100 | D60, D100 | 5 |
| Pulse type** | PAC | 5, 60, 150 | Ta5, Ta60, Ta150 | 5 |
| ***Sole (control)*** | | 0 | Ctr | 8 |

n: number of sole exposed to each combination of parameters; *: field strength was 100 V m$^{-1}$; ** pulse duration was 0.5 ms

**Table 5.2:** Overview of the parameter combinations to each of which ‘n’ cod were exposed. Each cod was exposed to one pulse ID, either in perpendicular or in parallel orientation toward the plate electrodes. The nominal pulse was a square PDC with a pulse duration of 250 μs and an exposure length of 2 s.

| **Parameter values** | | **Frequency (Hz)** | **Field strength ($V\ m^{-1}$)** | **Pulse ID's** | **n** |
|---|---|---|---|---|---|
| ***Wild cod (perpendicular)*** | | | | | |
| Frequency | | 40, 80, 120, 160, 200 | 100 | FEa40, FEa80, FEa120, FEa160, FEa200 | 1 |
| | | 60 | 150 | F60 | 3 |
| ***Wild cod (parallel)*** | | | | | |
| Frequency | | 60 | 150 | F60 | 3 |
| ***Wild cod (control)*** | | 0 | 0 | Ctr | 3 |
| ***Farmed cod (perpendicular)*** | | | | | |
| Frequency | | 40, 80 | 150 | F40, F80 | 4 |
| Pulse type | PBC | 40 | 200 | Tb40 | 4 |
| | PBC | 5, 80 | 150 | Tb5, Tb80 | 3 |
| ***Farmed cod (parallel)*** | | | | | |
| Frequency | | 80 | 200 | F80 | 4 |
| Pulse type | PBC | 5, 40, 80 | 200 | Tb5, Tb40, Tb80 | 4 |
| ***Farmed cod (control)*** | | 0 | 0 | Ctr | 11 |

n: number of cod exposed to each combination of parameters

## *Experimental design*

To ensure a homogenous electric field in the entire water column, two plate-shaped stainless steel electrodes of stainless steel were used. The electrodes were connected through two isolated copper conductors (Ø = 20 $mm^2$) with a laboratory pulse generator (LPG, EPLG bvba, Belgium) reaching a maximum output of 150 V, 280 A and 42 kW. The generator was also equipped with a feedback system to ensure that the output exactly matched the set values and was also checked by an oscilloscope (Tektronix TDS 1001B). Individual sole were placed in a flexible net positioned in the center of a 200 L aquarium of 0.42 m (W) x 1.2 m (L) x 0.42 m (H), between two plate electrodes (0.4 m x 0.4 m) in such a way that the fish was oriented either parallel or perpendicular to the electrodes (Figure 5.1). Wild and farmed cod were individually exposed in round PVC tanks (Ø = 4 m, 0.8 m water height) or rectangular polyester tanks (1.42 m x 6 m, 0.8 m water height). Cod were orientated to the electrodes by means of a PVC net in which the cod could move but not turn its body. The electrodes for perpendicular (0.4 m x 0.4 m) and parallel (0.4 m x 1m) exposures were placed at 1 m and 0.5 m respectively.

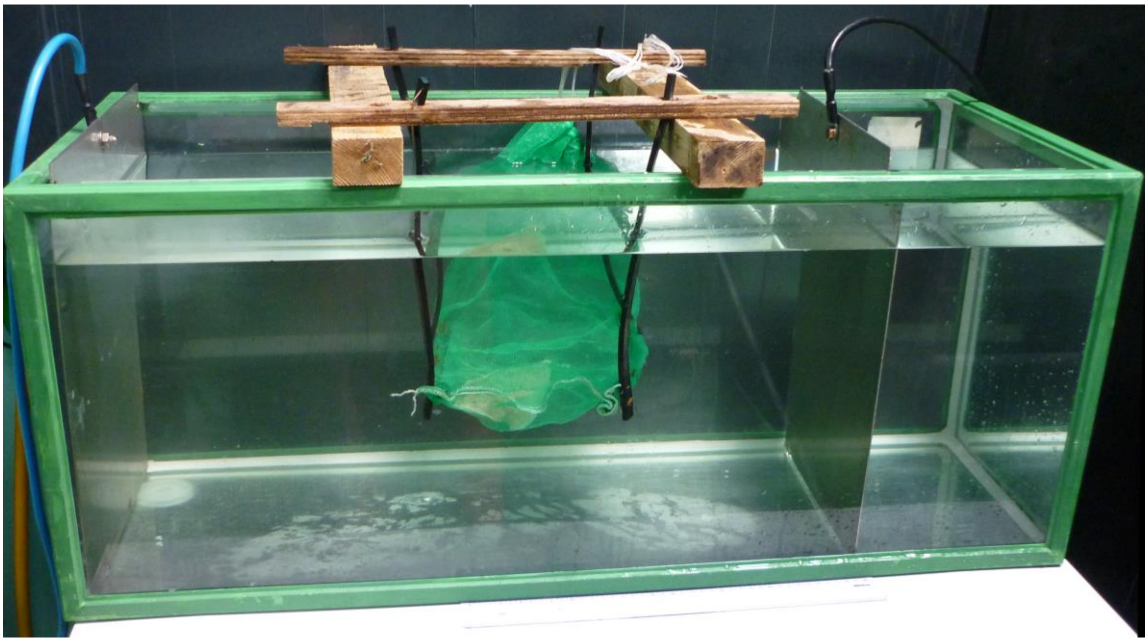


**Figure 5.1**: Homogenous set-up of a sole in a parallel orientation between two stainless steel plate-shaped electrodes prior to exposure.

The animals were fed both 24h prior and 24h after exposure as previously described. At the moment of exposure, randomly selected fish were transferred one at a time to the test tank and confined in the area between the two electrodes as described above. After the fish had calmed down and were oriented as envisaged (Table 5.1 and 5.2), the current was switched on. The reactions were recorded by means of a video camera (Sony ExmorR Handycam 12Mp) and special attention was given to tonic-clonic seizure reactions as described by Roth *et al.* (2003). The wild fish were tagged with labelled floy-tags when they were moved again to the housing tanks as described before. The tagging was done in the epaxial musculature ventrally to the first dorsal fin (cod). Each fish was exposed to only 1 electric stimulus. Control group animals were treated exactly the same as the exposed animals except for the exposure to the electric field, and divided randomly amongst the exposed animals. Sole were captured from the housing tanks 1 and 24 h after exposure to examine for petechiae, bleedings or lesions on the non-pigmented side. During the 2 week follow-up-period, the animals were monitored on a daily basis and mortality was recorded. Fourteen days post-exposure, all fish were sacrificed by immersion in an overdose of benzocaine or MS222 followed by cutting the gill arches. The animals were weighed and the total and standard lengths were recorded.

### *Macroscopic, histological and X-ray examination*

Following sacrifice, the animals were examined for external abnormalities including discolorations, haemorrhages and skin lesions. Subsequently, a full necropsy was performed. The gills, epaxial muscle, heart, liver, spleen, gut and kidney were sampled for histological examination. The samples were fixed in phosphate buffered formaldehyde (4%) for routine paraffin embedding and sectioning. Transversal sections of 5 µm thickness were cut with a microtome using the section transfer system (Microm, Prosan, Merelbeke, Belgium), stained with Haematoxylin/Eosin (HE) and examined light microscopically. In addition, the abundance of melanomacrophage aggregates (MMA) was scored semi-quantitatively for liver, kidney and spleen by observing at least 5 microscopic fields (20x power field) per tissue section. The following scoring system (0-5) was adopted based on the MMA presence per tissue section: 0 (MMA absent), 1 (<10%), 2 (10-20%), 3 (21-30%), 4 (31-40%), 5 (>40%).

To reveal spinal injuries or deformations, the carcasses were labelled and frozen for X-ray analysis. A dorsoventral projection (50 kV, 16 mAs) was taken from sole, while for cod two orthogonal radiographs (60 kV, 12.5 mAs), a dorsoventral and a lateral projection, were taken. For wild fish, radiography was performed at the Ghent University (EDR6 CANON, type CXDI-50G, flat panel detector, scintillator and amorphous silicon Sensor LANMIT 4, Santa Clara, California, USA), while farmed cod was X-rayed at the Nofima in Tromsø (Siemens Nanodor 2 X-ray machine).

## *Statistics*

The sum of the scores for melanomacrophage aggregates (MMA) found in the liver, kidney and spleen was used as response variable. To investigate the effect of different pulse regimens on the response variable, a linear fixed effects model was fitted incorporating the pulse regimes as categorical fixed effects, and also batch as an adjusting covariate. This was done since the different experiments of sole were performed in other seasons with different batches of fish, i.e. fish caught in the same area, which may affect the (physiological) reaction of the fish as a result of differences in experienced environmental stress. Additionally, if a particular pulse regime variable (i.e. E, D, S, T and L) was studied at different frequencies, the frequency was also included in the model as categorical fixed effect to adjust for it. For sole, the different pulse regime variable levels were compared pairwise between each other using Bonferroni's adjustment for multiple testing. For cod, however, the different pulse regimes were compared pairwise only with the control as there were no clear-cut comparisons allowing to compare a change in only one pulse regime variable, as was the case for sole. Finally, to study the overall relationship between frequency of the pulse and the total number of lesions, a linear regression model was fitted using total number of lesions as response variable and frequency as continuous fixed effect.

# Results

## *Dover sole*

Prior to exposure, all animals showed an escape response once they were placed in the exposure tank, but they ceased movements after 3-10 s when trapped in the corner of the netting. This motionless or slightly fin fluttering position on the bottom of the net was maintained until fish were exposed to electric pulses. When exposed to the lowest frequency (5 Hz), the muscles of the fish contracted on each pulse, giving an effect similar to the normal fin fluttering. The majority of the sole demonstrated a flight reaction, starting at the onset of the electric pulses, while less than 10% showed no displacement. When frequencies of 40 Hz or higher were applied, the muscles went into a cramp. The onset of this muscle cramp was characterized by distended opercula and a slightly curved body. As the exposure persisted or the pulse intensity was increased, the fish's body gradually bent in a U-form leading to a snout-to-tail position upon applying maximum field strengths, frequency or exposure time. One sole (PAC150, parallel) showed a small unilateral gill bleeding during exposure.

In the first seconds following exposure, a more variable reaction between individual fish was noticed, even when fish had been exposed to identical electric pulses. At 5 Hz, 20% of the fish remained motionless after exposure and another 20% showed very strong escape behaviour, while the others showed little to moderate escape behaviour. Fish which had displayed a cramp reaction clearly showed stronger post-exposure behaviour. Indeed, they either showed moderate to strong escape behaviour or displayed quivering during 2-5 s followed by a flight reaction. The latter was only seen at duty cycles above 3.75 % (Table 5.3). After being released back to the holding tanks, all sole (exposed and controls) immediately moved to the bottom corner of the tank and ceased movement.

**Table 5.3:** Number of sole exhibiting involuntary quivering (for 2-5 s) immediately after a 2 s exposure to square shaped pulses with the given pulse parameters in a homogenous electric field.

| **Pulse ID** | **Duty cycle (%)** | **Frequency (Hz)** | **Duration (µs)** | **Field strength (V m$^{-1}$)** | **Type** | **Quivering reaction** |
|---|---|---|---|---|---|---|
| ***Sole (perpendicular)*** | | | | | | |
| PAC150 | 3.8 | 150 | 250 | 150 | PAC | 0/3 |
| PBC150 | 3.8 | 150 | 250 | 150 | PBC | 0/3 |
| T150(1) | 3.8 | 150 | 250 | 150 | PDC | 2/3 |
| Ea200 | 5.0 | 200 | 250 | 100 | PDC | 3/3 |
| Eb200 | 5.0 | 200 | 250 | 200 | PDC | 3/3 |
| Db60 | 6.0 | 60 | 1000 | 150 | PDC | 0/3 |
| Db100 | 10.0 | 100 | 1000 | 150 | PDC | 3/3 |
| ***Sole (parallel)*** | | | | | | |
| F100 | 2.5 | 100 | 250 | 200 | PDC | 0/3 |
| F150 | 3.8 | 150 | 250 | 200 | PDC | 3/5 |
| PAC150 | 3.8 | 150 | 250 | 200 | PAC | 5/5 |
| F200 | 5.0 | 200 | 250 | 200 | PDC | 5/5 |
| Db100 | 10.0 | 100 | 1000 | 200 | PDC | 5/5 |

(1)Pulse ID T150 was a 150 Hz PDC of 150 V m$^{-1}$ and a pulse duration of 250 µs with an exposure time of 5 instead of 2 s.

**Table 5.4:** Statistical analysis of the cumulative scores of melanomacrophage aggregates (MMA) in spleen, liver and kidney in relation to the electric pulse used. This analysis was only done for fish of the same batch.

| **Parameter** | **Value** | **Estimate + s.e.** | **P** |
|---|---|---|---|
| ***Sole*** | | | |
| Field strength | 100 vs 200 V $m^{-1}$ | 5.00 ± 0.61 vs 5.17 ± 0.66 | 0.855 |
| Pulse duration | 0.1 vs 1 ms | 4.50 ± 1.05 vs 4.50 ± 1.05 | 1.000 |
| Pulse shape | quartersinus vs exponential | 4.83 ± 0.88 vs 5.00 ± 0.883 | 0.900 |
| Pulse type | PAC vs PBC | 1.33 ± 0.97 vs 2.33 ± 0.97 | 0.507 |
| Exposure time | 2 vs 5 s | 2.67 ± 0.47 vs 1.89 ± 0.47 | 0.264 |
| ***Cod*** | | | |
| Frequency | 40 vs 80 Hz | 0.50 ± 0.40 vs 0.75 ± 0.40 | 0.670 |
| Orientation | perpendicular vs parallel | 0.75 ± 0.40 vs 0.75 ± 0.40 | 1.000 |

During the 14 days follow-up period, one fish died on day 13 but it did not display any external or internal abnormalities. All other fish showed normal behaviour 1 h and 24 h after exposures with no external lesions. Subsequent necropsy showed no macroscopic lesions in any of the fishes. Histological examination of the gill, heart, muscle, liver, spleen, kidney and gut did not reveal any abnormalities. Furthermore, no significant effects of the electric pulse parameters on the abundance of MMA were found. The slope of the regression line fitted for frequency did not differ significantly from zero and the P-values of the analysis of all other parameters, given in Table 5.4, were higher than 0.05. Radiographic analysis revealed no fractures or dislocations that could be attributed to the exposure. In about one fifth of the animals, old spinal disorders were noted, which were either healed fractures, characterized by calcified nodules at the healed site (13.2%), or vertebral deformations (6.9%).

## *Atlantic cod*

Exposures to 5 Hz pulses elicited a flight response in all cod, irrespective of wild or cultured origin and small twitching muscle contractions could be distinguished at every pulse. All cod exposed to frequencies of 40 Hz and higher showed a cramp reaction accompanied with distended opercula during the entire exposure time. One wild cod (F60, perpendicular) displayed black coloration of the skin under the 2$^{nd}$ dorsal fin as illustrated in Figure 5.2, which remained visible during the 14 days follow-up period.

Immediately after exposures, two different responses were noticed. At low electric loads (reduced duty cycles and field strengths), fish swam agitatedly and were trying to flee. At higher electric loads of high duty cycles and/or high field strengths fish showed an epileptiform response (Table 5.5). The latter was characterized by a succession of several reactions. When the electric stimulation ceased, the cramped status of the muscles remained whilst the opercula were distended. Some fish turned on their side and after 3-5 s their muscles started quivering rapidly leading to small contractions evolving within 3 s into a vigorous shaking of the body. At that time, the fish appeared in an unconscious state as indicated by no reaction to stimuli, loss of equilibrium and ceased ventilation. This was sometimes accompanied by regurgitating and/or eggs or sperm release. This clonic phase changed gradually into a tonic phase (a slight shiver) in the next 30 s while the majority of the fish were still lying on their side remaining unconscious. After ± 2-3 min, respiration slowly recovered and weak signs of reactivity occurred where after the fish were released into the holding tanks. Within 10 min post-exposure, most of the fish were breathing normally and showed little swimming activity with partial equilibrium loss and a weak reactivity to tactile stimuli which persisted during the first hours following exposure. All these fish survived and showed normal behaviour 24h post exposure.

Gross lesions associated with electric pulses were observed in the one cod showing local black discoloration after exposure. During autopsy, an internal bleeding was observed in the areas cranial to the black discoloration (Figure 5.2), which could be attributed to an acute vertebral compression between the 20th and 25th vertebra after X-ray analysis (Figure 5.3). X-ray analysis uncovered chronic deformities and healed bone fractures in respectively 7.1% and 14.3% of the wild cod. In farmed cod, 2.9% and 43% of the individuals revealed healed bone fractures and malformed, fused or compressed vertebrae, respectively. In 25% of the farmed cod, including both exposed and control animals, multifocal white spots were discerned macroscopically on the gills.

**Figure 5.2:** Black discoloration (top) and focal haemorrhage (bottom) ventral to the 2nd dorsal fin 14 d post exposure associated with spinal column compression of the 20th – 25th vertebrae in a wild cod**.**

**Table 5.5:** Number of cod exhibiting strong epileptiform reactions immediately after a 2 s exposure to square shaped pulses with the given pulse parameters.

| **Pulse ID** | **Duty cycle (%)** | **Frequency (Hz)** | **Duration (µs)** | **Field str. (V m$^{-1}$)** | **Pulse type** | **Epileptiform reaction** |
|---|---|---|---|---|---|---|
| ***Wild cod (perpendicular)*** | | | | | | |
| F40 | 1.0 | 40 | 250 | 100 | PDC | 0/1 |
| F60 | 1.5 | 60 | 250 | 150 | PDC | 2/3 |
| F80 | 2.0 | 80 | 250 | 100 | PDC | 0/1 |
| F120 | 3.0 | 120 | 250 | 100 | PDC | 1/1 |
| F160 | 4.0 | 160 | 250 | 100 | PDC | 1/1 |
| F200 | 5.0 | 200 | 250 | 100 | PDC | 1/1 |
| ***Wild cod (parallel)*** | | | | | | |
| F60 | 1.5 | 60 | 250 | 150 | PDC | 0/3 |
| ***Farmed cod (perpendicular )*** | | | | | | |
| PBC5 | 0.1 | 5 | 250 | 150 | PBC | 0/3 |
| F40 | 1.0 | 40 | 250 | 150 | PDC | 0/4 |
| PBC40 | 1.0 | 40 | 250 | 200 | PBC | 4/4 |
| F80 | 2.0 | 80 | 250 | 150 | PDC | 4/4 |
| PBC80 | 2.0 | 80 | 250 | 150 | PBC | 3/3 |
| ***Farmed cod (parallel)*** | | | | | | |
| PBC5 | 0.1 | 5 | 250 | 200 | PBC | 0/3 |
| PBC40 | 1.0 | 40 | 250 | 150 | PBC | 0/4 |
| PBC80 | 2.0 | 80 | 250 | 200 | PBC | 3/3 |
| F80 | 2.0 | 80 | 250 | 150 | PDC | 2/4 |

Histological examination of the internal organs did not reveal any abnormalities linked to the electric exposures. Both exposed and control wild cod showed lymphoid follicular hyperplasia in 77% of the spleens examined and granulomas were found in 23% of the liver and 31% of the hearts. The percentage of wild cod with MMA scores higher or equal to 1 in the spleen, kidney and liver was 67%, 33% and 67% for the control and 91%, 43% and 20% for the exposed animals, respectively. No MMA were found in the liver of farmed cod, and the percentage of animals with MMA in the spleen and kidney was 0% and 33% for the control and 8% and 24% for the exposed animals, respectively. No statistical differences were found when comparing any of the pulse parameter treatments with the control animals (Table 5.5). This analysis was not done for wild cod as animals from different origin or a too low number of animals were used. Histological examination of the gill lesions demonstrated multifocal hyperplasia of gill lamellae with an intralesional protozoan parasite.

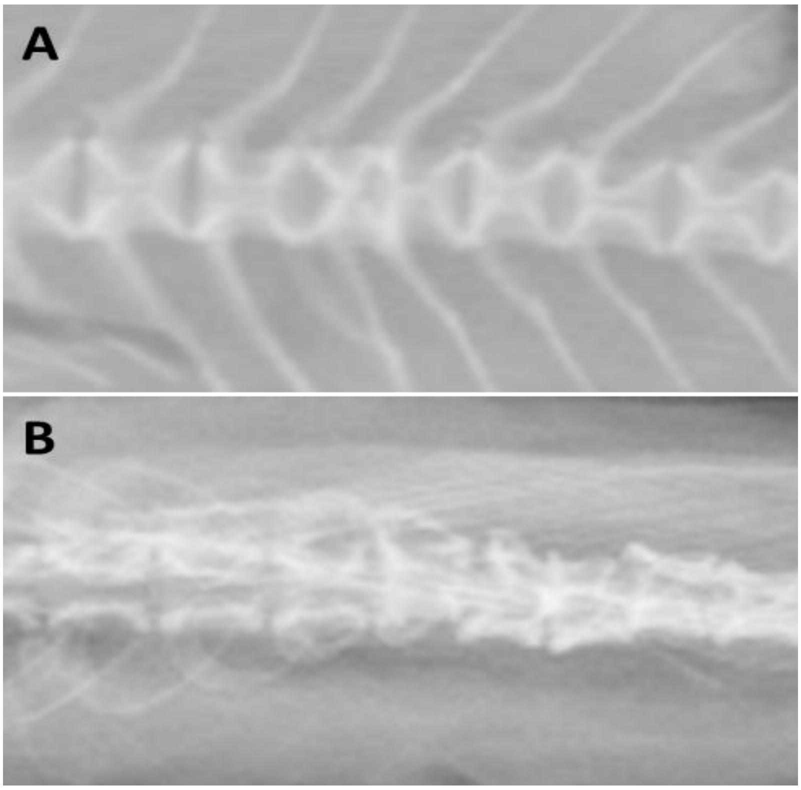


**Figure 5.3:** Lateral-lateral (A) and dorso-ventral (B) X-ray view demonstrating the compression of the 20th - 25th vertebrae in wild cod shown in Figure 5.2.

# Discussion

## *Reaction of the fish*

The flight reaction observed **during exposure** to frequencies up to 20 Hz has previously been reported for both Atlantic cod and sole (Stewart, 1973; Polet *et al.*, 2005a). Additionally, these authors reported higher incidences of virtually no response up to 75% when flatfish were buried in the substrate. At frequencies above 40 Hz, both species showed a cramp reaction, which is a rapid succession of muscular contractions, resulting in immobility. Cramp reactions induced by electric fields were not only previously described for sole (Stewart, 1977) and cod (De Haan *et al.*, 2009b & 2011), but in other marine fish such as plaice (*Pleuronectes Platessa* L.) and lemon sole (*Microstomus Kitt* W.) (Stewart, 1977), seabass (*Dicentrarchus labrax* L.) (D'Agaro & Stravisi, 2009) and dogfish (*Scyliorhinus canicula* L.) (De Haan *et al.*, 2009a), as well as in marine invertebrates such as brown shrimp (Soetaert *et al.*, 2014) and numerous fresh water species (McBary, 1956; Snyder, 2003a). The threshold frequency inducing a cramp reaction in vertebrate species is reported to be around 20 Hz and dependent upon the other pulse parameters (Snyder, 2003a).

The reaction of the fish immediately **after exposure** was most often an escape response. This has also previously been described for invertebrates (Soetaert *et al.*, 2014), cod (De Haan *et al.*, 2009a & 2011) and flatfish (Stewart, 1973, 1975b, 1977). In general the escape response is directly proportional to the intensity of the electric field (Soetaert *et al.*, 2014; Stewart, 1974, 1975b & 1977), which was confirmed in our study. This indicates that electric pulses could also be optimized to induce an escape reaction in sole, rather than the cramp reaction which is used nowadays. Stewart (1973, 1975b & 1977) reported a jump-and-flight reaction in over 80% of the sole exposed to 20 Hz bursts of 1 s alternated with 1 s breaks. Further optimization of electric pulse parameters, aiming for this startle response may enable a herding effect of sole, which could offer major opportunities in rendering otter trawls more efficient in catching sole. When exposed to high duty cycles and/or field strengths, strong epileptiform reactions were observed in cod and quivering behaviour in sole. The threshold of epileptiform reactions is related to various factors such as species, size, and the physiological state of the individual neurons in the brain (Purpura *et al.*, 1972). Indeed, studies have shown that large fish can more easily be immobilized by electric pulses than small individuals because larger fish react to lower electric field strengths (Bird & Cowx, 1993; Dalbey *et al.*, 1996; Emery, 1984; Reynolds, 1986; Zalewski & Cowx, 1990). More recently, Dolan and Miranda (2003) showed that, regardless of the fish species, fish volume is the best size descriptor and total wet weight could be used as an indirect measure for fish volume. They demonstrated that the peak power needed to immobilize fish, decreased rapidly with increasing fish volume in small fish, but decreased slowly for fish larger than 75-100 $cm^3$. Our study seems to confirm this hypothesis: the quivering behaviour of sole (162 ± 59 g) was observed at duty cycles of about two times higher than the those on which epileptiform seizures were induced in wild (546 ± 133 g) and farmed cod (4166 ± 723 g). Moreover, all epileptiform stages were seen in cod, where sole only showed short quivering, which again highlights the vulnerability of cod to electric pulses.

**Epileptiform seizures** are observed in all vertebrates (Purpura *et al.*, 1972; Servit & Strejckova, 1970). They occur when the nervous system is overloaded by a stimulus, whether electric, chemical or other (Delgado-Escueta *et al.*, 1986; Penfield & Jasper, 1954). The persistence of the muscle response is caused by rhythmical discharge impulses of the neurons after cessation of stimulation often called 'afterdischarge' (Lothman & Williamson, 1992). Hence, the post-exposure muscle response does not result from stimulation of local nerves, but from (over)stimulation of the nervous system (Sharber & Black, 1999). The occurrence of electrically-induced epileptiform seizures depends on different parameters. It occurs more likely at higher field strengths or frequencies and longer pulse durations or exposure times (D'Agaro & Stravisi, 2009; Lothman & Williams, 1992; Nordgreen *et al.*, 2008; Roth *et al.*, 2003 & 2004; Sharber *et al.*, 1994; Sharber & Black, 1999; Shigeto *et al.*, 2013; Weiner, 1988). Frequency is said to be the predominant factor in fish (D'Agaro & Stravisi, 2009; Roth *et al.*, 2004; Sharber & Black, 1999; Weaver *et al.*, 1974) as well as in other vertebrates (Lothman & Williamson, 1992; Shigeto *et al.*, 2013).

This is attributed to the fact that frequency can change the brain impedance, and thus the current reaching the brain which affects the response of neurons (Finlay *et al.*, 1978). The clear promoting effect of frequency on epileptiform seizures is also observed in this study (Table 5.3 & 5.4: parallel exposures). Besides, the tests performed with sole with increased pulse duration (Table 5.3: Db100 vs F100) and exposure time (Table 5.3: T150 vs PAC150 & PBC150), also promoted the incidence of epileptiform behaviour, as described by Roth *et al.* (2003). However, more elaborate research is required to fully unravel the effect on and interaction of the pulse parameters with epileptiform reactions, which was not the objective of this study. The threshold for epileptic seizures may also be affected by the orientation of the fish. The nervous system is most stimulated in perpendicular orientation, where the potential difference over the spinal cord is largest (Snyder, 2003a). However, the above mentioned hypothesis cannot explain the stronger epileptiform reactions observed in cod exposed parallel to the electrodes. In that case, the potential difference over the spinal cord, positioned on equipotentials parallel to the electrodes, would be insufficient to elicit the observed reaction. This indicates that stimulation through segmental nerves or direct stimulation of the brain may play a more decisive role, as it has been demonstrated in rats (Shigeto *et al.*, 2013). Furthermore, the reactions observed in cod at the given frequencies were not previously reported in heterogenous set-ups and may be promoted by the homogenous set-up used. These findings underscore the need for a better understanding of fish physiology in relation to electric fields to enable to interpret and explain the encountered results, and to assess the impact of morphologic differences between flatfish and roundfish.

## *Injuries*

In the current experimental set-up, the different pulse parameters were altered to reveal side-effects. Every pulse parameter studied was tested at different frequencies since a positive correlation between pulse frequency and injury rate was previously reported (Dolan & Miranda, 2003; Schreer *et al.*, 2004; Sharber *et al.*, 1994). As a first result, no 14-day mortality was observed in the sole or cod studied. As a first result, no 14-d mortality was observed in cod, whereas one sole without observable lesions was found death on day 13. This accords with previous experiments in which fish was exposed below immobilization threshold (Dolan & Miranda, 2003), but differs from studies in which fish, forced into cramp, were often found with mortality rates ranging from 0 to 30 % (Sharber & Carothers, 1988; Sharber *et al.*, 1994; Dolan *et al.*, 2002; Dolan & Miranda, 2004; Schreer et al, 2004; De Haan *et al.*, 2009b). However, as the rates of mortality and injuries are not always directly related (Dalbey *et al.*, 1996; Dolan *et al.*, 2002; Dolan & Miranda, 2004; Miranda & Kidwell, 2010), the exposed animals were further examined for external and internal as well as macroscopic and microscopic injuries.

In one of the 39 cod exposed to a cramp pulse (≥40 Hz), a spinal column dislocation occurred during exposure. This was characterized by black discoloration of the skin located caudal of the lesion. Sharber and Black (1999) suggested that dark discoloration of the skin is due to the dilution of skin melanophores, possibly as a result of sympathetic nerve damage. As nerval damage may result from spinal fractures or dislocations, this may explain the dark discoloration of the tail caudal of the spinal lesion. Radiographical examination revealed a compression of the 20th-25th vertebral body. These findings are in accordance with those previously reported (De Haan *et al.,* 2011, Van Marlen *et al.*, 2014). However, the low injury rate for cod in our study was unexpected, since, even with lower field strengths, incidences of vertebral injuries as high as 70% in farmed cod (De Haan *et al.* 2009a & 2011) and rainbow trout (Dalbey *et al.*, 1996; Schreer *et al.*, 2004; Sharber *et al.*, 1994; Sharber & Carothers, 1988) have been described. It is unclear whether this is due the experimental setup (homogenous vs heterogenous field strength) or intrinsic properties of the fish, such as body musculature, physiology and morphology. Spinal injuries are believed to result from strong bilateral cramp contraction of the white spinal muscles induced mostly by sudden changes in voltage (Snyder, 2003b). This could explain the decrease in occurrence of spinal injuries when a cramp reaction is avoided by using direct current, low-frequency pulsed direct current (<30Hz), or specially designed pulse trains (Dolan & Miranda, 2004; Snyder, 2003b). In contrast, spinal injuries were not observed in sole and, as far as we know, have not yet been recorded in literature concerning flatfish (Stewart, 1973 & 1977; Stewart & Copland, 1976). In addition, spinal injuries in flatfish were not reported in the field either, even though commercial electrotrawls targeting flatfish are frequently operating in the North Sea. Hence, all available information indicates that the tolerance for (spinal) injuries observed in this study for sole, may also apply for other adult marine flatfish species. This may be related to the relatively smaller amount of white muscles in flatfish compared to round fish. Besides, differences in anatomy can also play a role: while flatfish morphology results in an arched back during a cramp reaction, distributing the force more equally over the spine, cod showed a straight contraction in which the contraction powers of the left and right musculature are reinforcing one another.

During exposure, minor gill haemorrhage was observed in one of the 146 sole. This animal survived and 14 days after the exposure no microscopic gill lesions were observed. Another sole died 13 days after exposure, but the cause of death could not be ascertained. Gross and histological examination of all other sole as well as all cod, did not reveal lesions attributable to electric pulses. No sign of hypoxic damage, caused by possible cardiac arrest during exposure (Schreer *et al.*, 2004), nor respiration arrest during epileptiform seizures were found. To demonstrate a possible response to environmental stress (Agius and Roberts, 2003), the size and frequency of MMA were scored histologically. Wild cod showed much more MMA proliferation than farmed fish, which is probably due to pollution or contaminants in (coastal) water as demonstrated for Atlantic tomcod, red mullet and seabass (Carrasson *et al.*, 2008; Couillard *et al.*, 1999; Giari *et al.*, 2007). However, no correlation between the abundance of MMA and exposure rate was found. A similar outcome has been observed in exposure experiments with brown shrimp and ragworm (Soetaert *et al.*, 2014).

# Conclusion

This study demonstrates that adult cod are sensitive to exposure to electric pulses in a homogenous set-up, in the sense that epileptiform reactions are evoked, and confirms that spinal injuries may occur during a cramp-reaction. However, the obtained injury rate was much lower than reported in previous research. In contrast, sole only demonstrated a short quivering when exposed to the highest electric loads without the occurrence of lesions, indicating the tolerance of adult sole.

# CHAPTER 6

# SENSITIVITY OF COD TO ELECTRIC PULSES: AN INTRIGUING PHENOMENON REMAINING UNRAVELLED

Adapted from:

Soetaert, M., de Haan, D., Verschueren, B., Decostere, A., Puvanendran, V., Saunders J., Polet, H., & Chiers, K. 2015. Atlantic cod (*Gadus morhua* L.) show highly variable sensitivity for electric-induced spinal injuries.
Accepted with revisions in Marine and Coastal Fisheries, November 2015.

# Abstract

Pulse trawling is the most promising alternative for conventional beam trawls targeting sole, but, unfortunately, due to the electric fields created by electrotrawlers, spinal injuries are reported in gadoid round fish such as Atlantic cod *Gadus morhua*. Electric-induced injuries were also observed in freshwater Salmoninae and varied depending on the pulse settings, the electrode set-up and environmental conditions and the fish stocks used. This study aimed to investigate possible variability in the occurrence of electric-induced in cod. Four groups of cod, originating from a different wild or farmed stock, were exposed similarly to pulses used by electrotrawls targeting sole. Effects were analyzed based on behaviour, mortality and lesions up to 14 days after exposure and morphological characteristics such as and size, somatic weight, muscularity, number of vertebral bodies and vertebral mineral contents of animals were compared between different cohorts. Second, the influence of parameters such as water temperature, electrode diameter, pulse type and amplitude were tested. Electrode diameter and pulse amplitude showed a positive correlation with the intensity of the fish's reaction. However, the present experiments also confirmed that marine fish such as cod also show a variable vulnerability, with an injury rate varying between 0 and 70% after an (almost) identical exposure near the electrode. This indicates that these injuries are not only determined by the pulse parameter settings, but also by subtle fish-specific parameters. Although the absence of a sensitive group of cod did not enable the elucidation of the decisive parameter, the effect of physiological as morphological parameters such as intervertebral ligaments and rearing conditions during early life merit further attention in future research.

# Introduction

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

Studies investigating the side effects of these electric pulses revealed varying results for different marine species. Indeed, exposure of invertebrates (Soetaert *et al.*, 2014) or sole (Soetaert *et al.*, 2015b) or seabass (Chapter 7) to the electric pulses used in the field did not elicit mortality nor lesions. Gadoid roundfish such as whiting (*Merlangius merlangus* L.) and Atlantic cod (*Gadus morhua* L.) on the other hand displayed spinal injuries (van Marlen *et al.*, 2014; de Haan *et al.*, 2011; Soetaert *et al.*, 2016), albeit with different levels of severity in terms of number of animals affected. These spinal injuries result from powerful convulsions of the body musculature when exposed to electric pulses (Snyder, 2003). Catch comparisons in the field demonstrated spinal injuries in 4 out of the 45 cod caught by pulse trawlers (van Marlen *et al.*, 2014), while 70% of cod (> 0.3 m) experimentally exposed near the electrodes' conductors to field strengths ≥ 37 V m$^{-1}$ were impacted (De Haan *et al.*, 2011). In the same study, juvenile cod (0.12 - 0.16 m) exposed to high field strengths of 250-300 V m$^{-1}$ did not reveal vertebral injuries (de Haan *et al.,* 2011). In a recent lab study, no spinal injuries were observed when cultured and wild-caught cod were exposed to similar pulses in a homogenous electric field (Soetaert *et al.*, 2015b). These results suggest that the extent to which electric pulses exert a negative impact depends not only on the electric pulse parameters, but possibly also on the fish and experimental conditions used. Such variability is also reported in freshwater electrofishing, with particularly Salmoninae showing high but variable injury rates (Snyder, 2003). Since gadoid roundfish show similar high numbers of vertebrae (± 52 vertebrae), it can be hypothesized that such variability may also occur in cod. Although electrotrawl induced spinal injuries are a major concern, no research has been done to compare the sensitivity of different stocks in marine species. Moreover, studies correlating morphological and physiological parameters to injuries in marine species are non-existing.

Therefore, the goal of the present study is to aims assess the extent of this variability in cod, starting from the cramp pulse used in commercial electrotrawls. Therefore, wild cod and farmed cod of different stocks, showing a different size, morphology and condition, were exposed to the same electric pulses and electrode set-up. Subsequently, behaviour, mortality and lesions were recorded up to 14 days after exposure and size. During and after autopsy, somatic weight, muscularity, number of vertebral bodies and vertebral mineral contents of animals of these groups were examined to reveal possible correlations with the observed reactions. Additionally, the effect of water temperature, pulse type, pulse amplitude, the fish's orientation, the presence of an inductor and the electrode diameter were tested. These environmental factors are also variable in electrotrawls targeting sole and may therefore provide a better insight in how they may affect the variability in commercial fishing practice.

# Materials & Methods

## *Animals and housing facilities*

To examine the variability between different stocks, wild and farmed Atlantic cod of different origin where included and compared. A first group (W) consisted of wild cod (0.39 ± 0.05 m) caught with hook and line in the North Sea at the Blight bank wind farm 25 miles off the Belgian coast. They were housed as described previously (Soetaert *et al.*, 2015b) and acclimated for 3 months. A second group (F1) comprised 2.5 years old farmed cod (0.40 ± 0.02 m) obtained from the IMR's research station, Austevoll, Norway. This group was included as a reference group for the experiments done by de Haan *et al.* (2011), who performed their experiments in this research station using cod of the same stock. The cod were transferred 2-3 days prior to the exposure to the electric pulses from sea cages to an open air circular tank of 6 $m^3$, continuously supplied with fresh seawater, pumped from the adjacent fjord. This tank was covered with a tipi in plastic canvas to reduce the light intensity and minimize external perturbation. No food was provided during the days pre-exposure. A third group (F2) of cod (0.71 ± 0.04 m) was taken from the 4-years old brood stock of the Norwegian Cod Breeding Centre, NOFIMA Institute, Tromsø, Norway. This brood stock has been intensively ennobled the past decade for commercial farming and may therefore show significant morphological differences with the F1 fish. Moreover, they were much larger than the previous two groups. These animals were kept in sea cages during six months (July - January) and moved to large circular tanks of 23 $m^3$ supplied with fresh seawater during spawning season (February - June). This group was divided in two subgroups (F2a, F2b). The F2a group consisted of randomly chosen gravid cod. Animals of the F2b group were first selected based on tail muscularity, with the tail circumference cranial to the tailfin measuring at least 20 cm, and then randomly divided over the different treatments. All F2-animals were fed daily with dry pellets (Vitalis Cal, Skretting, Spain). The fourth group (F3) of cod

(0.45 ± 0.03 m) encompassed a 2-years old offspring of F2 and showed a similar size as the F1 fish. They were housed in the Aquaculture Research Station (Tromsø, Norway) in circular tanks of 2 $m^3$ continuously supplied with fresh seawater from the adjacent fjord. These fish were fed daily (Amber Neptune 300, Skretting, Averoy). All these farmed animals were intensively reared in tanks during the first ten months of their life before they were transferred to sea cages. These experiments were approved by the Belgian (ID 2011/170) and Norwegian Animal Welfare and Ethical Committees (ID 5183).

### *Experimental set-up and variables*

Fish of the W, F1 and F3 groups were tagged with individually coded floy-tags at the onset of the first dorsal fin. Fish from the F2 group were individually identified for the breeding program by means of PIT-tags. In all experiments, animals were taken from a pre-exposure housing tank and exposed one by one in an identical exposure tank. Thereafter they were moved to an identical post-exposure housing tank, housed together and monitored during 2 weeks, except for the F1 animals which were euthanized 1-24 h post exposure. During exposure, the reaction of the animal was recorded with a digital video camera. A total of 25 wild cod, 80 F1 cod, 27 F2a, 35 F2b and 38 F3 cod were used, of which 5, 10, 5, 5 and 10 animals were used as controls, respectively (Table 6.1). These control animals were included to compare possible mortality or histological abnormalities between exposed and non-exposed animals. They were chosen randomly and handled similarly but were not exposed to electric pulses.

The experiments were set-up similarly for all animals (Table 6.1), but performed at a different time and water temperature (Tw) in the same year (2013): group W: December (Tw = 15.0°C), F1: October (Tw = 7.5°C), F2a: April (Tw = 3.5-4.0°C), F2b: June (Tw = 4.5-4.9°C), and F3: May (Tw = 4.4-5.5°C) (Table 6.1). The salinity remained constant at 34 ppt. All experiments were performed with wire-shaped electrodes resulting in a heterogeneous electric field, but two different electrode configurations were used. First, the same wire-shaped electrodes of a Delmeco pulse trawl system for flatfish was used as described by De Haan *et al.* (2011). These consisted of two copper conductors (0.18 m, Ø26 mm) lifted from the bottom by 2 PVC discs (Ø70 mm, 1 cm width) at both ends and separated by an insulator of 0.57 m, as illustrated in Figure 6.1. However, the discs were removed during all experiments with F2 and F3 fish (Figure 6.1). The mutual distance between the electrode cores was 0.325 m. Second, electrodes of the Marelec pulse trawl system for shrimp were used in two experiments with F3 fish (pulse IDs IX & X; Table 6.1). These consisted of stainless steel with a copper core without any insulation (length = 1 m, Ø= 12 mm). The electrodes were placed on the same mutual distance of 0.325 m, in contrast to the 0.7 m field configuration.

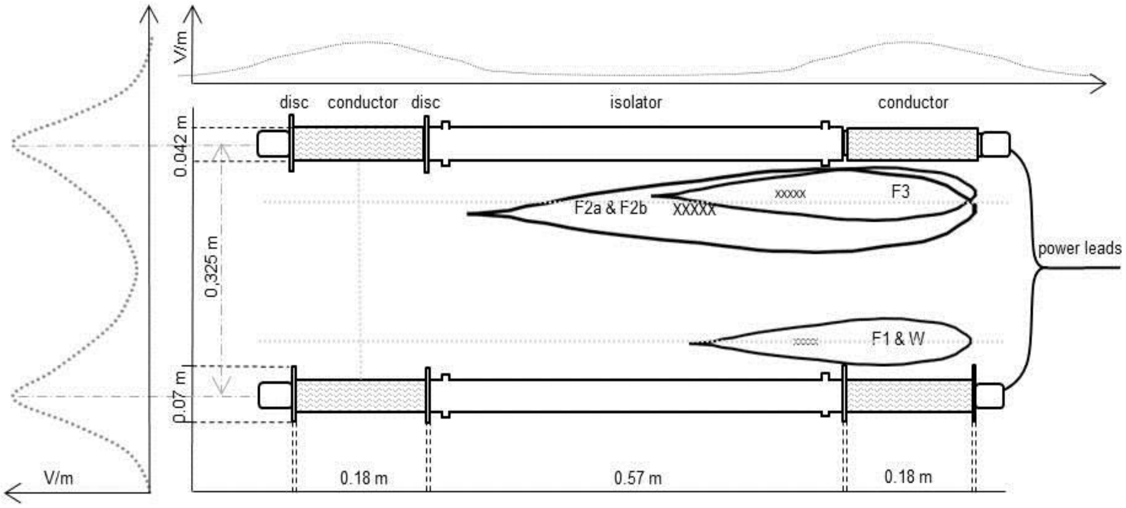


**Figure 6.1**: Schematic top view of the principal set-up with Delmeco electrodes with (lower electrode) and without discs (upper electrode) at both ends of the conductor. The dashed lines indicate the size of each part. The horizontal dotted black graph gives the course of the field strength (V m$^{-1}$) measured at the dotted grey line, 55 mm from the electrodes. The vertical dotted black graph gives the approximate exponential course of the field strength measured perpendicular to the centre of the conductor. Localization of spinal injuries is indicated with X.

To compare the electric field generated by both electrode configurations, an approaching simulation of the electric field strengths around and in the cod was made with Finite Element Method Magnetics (FEMM) (Meeker, 2006), a free software packet solving 2D planar and axisymmetric problems in low frequency magnetics and electrostatics. The electrodes' potential difference used was 60 V, while the conductivity of the copper electrodes, the seawater and the sand was set on 58 x $10^6$, 4.2 and 1 S $m^{-1}$, respectively. A value of 0.0115 S $m^{-1}$ was adopted for fish conductivity, as recommended by Miranda and Dolan (2003).

During the reference exposures of all groups (pulse IDs I & II), cod were exposed in the near field configuration as described by De Haan *et al.* (2011). Therefore, cod were placed with its longitudinal body axis as close to the conductor as possible, while the tip of its snout was located at the front of the first conductor by fixing the animal in a triangular V-shaped cage made of PVC netting according to de Haan *et al.* (2011) (Figure 6.1). The bottom of the V-shaped cage was attached near electrode. The fish could swim freely, but were forced downward using a plate of equivalent mesh material on a stick. Immediately afterwards, the pulse was triggered. As soon as a response of the fish was felt, the fish was no longer forced downward to minimize the influence on the reaction during and after exposure (de Haan *et al.*, 2011). In one trial with F2b fish (VII), an exception was made and the animals were oriented perpendicularly with the snout above one conductor and the tail above the other. In part of the experiments with F1 cod (II, V), an inductor was used in series with the leads to the electrodes, to simulate the effect of inductive elements in the discharge circuit (switching transformers, cabling). This inductor prolonged the rise time of the pulse, which was defined as the time interval between the onset of the pulse and its maximal amplitude, from 50 to 250 µs.

## Pulse parameters

The amplitude (U, [V]) of the pulses modulated by the pulse generators based on the potential difference over the electrodes. Other pulse parameters were the frequency (F, [Hz]), the number of pulses per second; electric current (I, [A]), pulse duration (D, [µs]), the duration of a single pulse, and the pulse type (T). The rise time (R, [µs]) was defined as the time interval between the onset of the pulse and its maximal amplitude. Both pulse types used in electrotrawls targeting sole were used: a pulsed alternating current (PAC) with a positive and negative part in each pulse, and a pulsed bipolar current (PBC) with alternating a positive and a negative pulse. The nominal pulse had a square shape and a pulse duration of 250 µs, although this was strongly affected by the impedance of the inductor, electrodes and power leads. The exposure time was 1 s in the experiments with animals of the F1 group, and 2 s in all others, which is approximately 2 times longer than the field situation. The duty cycle (dc, [%]), i.e. the ratio (in %) between the active pulse duration (D in s) and the period of a pulse cycle (1/F in s), is given as measure for the time during which electric current was running. Finally, the maximal electric power (P, [W]) released by the electrodes in the water was calculated by multiplying the peak amplitude in voltage with the peak current in Ampère. An oscilloscope (Tektronix TDS 1001B) was used to measure the effective potential difference, pulse shape and rise time on the conductors. To determine the electric current, an additional Rogowski shunt was employed. The measured values are listed in listed in Table 6.1.

Table 6.1: Overview of experimental design, pulse settings and obtained results. All animals were fixed parallel near the conductors during exposure. Electric stimuli were produced by either the Delmeco generator used on commercial electrotrawls targeting sole or by the Labo Pulse Generator (LPG). Salinity was 34 ppt at all times while the water temperature (Tw) at the moment of exposure is recorded. The pulse parameters given are: exposure time (t, [s]), frequency (F, [Hz]), peak amplitude (U, [Vp]), peak current (I, [Ap]), pulse type (T), pulse duration (D, [µs]), rise time (R, [µs]), duty cycle (dc, [%]) and peak power (P, [kWp]). The pulse type was Pulsed Bipolar Current (PBC) or Pulsed Alternating Current (PAC). The percentage of animals that demonstrated epileptiform seizures (epi), paravertebral haemorrhages (he) and spinal injuries (inj) are listed.

| | | Set-up | | | Pulse settings | | | | | | | | | | | Effect (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | Pulse ID | generator | inductor | electrode | Tw (°C) | t (s) | F (Hz) | U (Vp) | I (Ap) | T | D (µs) | R (µs) | dc (%) | P (kWp) | # fish | epi | he | inj |
| W | I | LPG | No | Delmeco | 15.0 | 2 | 80 | 60 | 72 | PBC | 310 | 40 | 2.48 | 4.32 | 20 | 0 | 5 | 5 |
| | CTR | | | | | | | | | | | | | | 5 | 0 | 0 | 0 |
| F1 | I | LPG | No | Delmeco | 7.5 | 1 | 80 | 60 | 76 | PBC | 310 | 40 | 2.48 | 4.58 | 20 | 0 | 0 | 0 |
| | II | Delmeco | Yes | Delmeco | 7.5 | 1 | 80 | 60 | 78 | PBC | 270 | 220 | 2.16 | 4.68 | 23 | 0 | 4 | 0 |
| | III | Delmeco | No | Delmeco | 7.5 | 1 | 80 | 60 | 82 | PBC | 270 | 60 | 2.16 | 4.92 | 7 | 0 | 0 | 0 |
| | IV | LPG | No | Delmeco | 7.5 | 1 | 80 | 120 | 150 | PBC | 310 | 60 | 2.48 | 18.00 | 10 | 80 | 0 | 0 |
| | V | LPG | Yes | Delmeco | 7.5 | 1 | 80 | 120 | 130 | PBC | 350 | 250 | 2.80 | 15.60 | 10 | 30 | 30 | 20 |
| | CTR | | | | 7.5 | | | | | | | | | | 10 | 0 | 0 | 0 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F2a | I | LPG | No | Delmeco | 3.5 | 2 | 80 | 58 | 80 | PBC | 310 | 60 | 2.48 | 4.64 | 16 | 0 | 0 | 0 |
| | VI | LPG | No | Delmeco | 3.5 | 2 | 40 | 58 | 80 | PAC | 550 | 80 | 2.20 | 4.64 | 6 | 0 | 0 | 0 |
| | CTR | | | | 3.5 | | | | | | | | | | 5 | 0 | 0 | 0 |
| F2b | I | LPG | No | Delmeco | 4.5 | 2 | 80 | 60 | 80 | PBC | 310 | 60 | 2.48 | 4.80 | 10 | 0 | 0 | 0 |
| | VII(a) | LPG | No | Delmeco | 4.5 | 2 | 80 | 60 | 80 | PBC | 310 | 60 | 2.48 | 4.80 | 10 | 0 | 0 | 0 |
| | VIII(b) | LPG | No | Delmeco | 10 | 2 | 80 | 60 | 80 | PBC | 310 | 60 | 2.48 | 4.80 | 10 | 0 | 0 | 0 |
| | CTR | | | | 4.5 | | | | | | | | | | 5 | 0 | 0 | 0 |
| F3 | I | LPG | No | Delmeco | 4.4 | 2 | 80 | 60 | 80 | PBC | 310 | 60 | 2.48 | 4.80 | 14 | 0 | 0 | 0 |
| | IX | LPG | No | Marelec | 4.4 | 2 | 80 | 61 | 165 | PBC | 260 | 120 | 2.08 | 10.07 | 7 | 0 | 0 | 0 |
| | X | LPG | No | Marelec | 4.4 | 2 | 40 | 60 | 165 | PAC | 520 | 120 | 2.08 | 9.90 | 7 | 0 | 0 | 0 |
| | CTR | | | | 4.4 | | | | | | | | | | 10 | 0 | 0 | 0 |

(a)cod was exposed perpendicularly with its head above the first conductor, and its tail above the other conductor; (b)cod was acclimated during 14d at 10°C and exposed at 10°C under pulse ID I settings.

## *Post exposure examination*

After exposure, external lesions, aberrant behaviour and mortality were recorded 1h, 2h and 24 h post exposure (group F1) and 1h, 2h and each 24h for 14 days (all other groups) by visual inspection of the fish, preferably during feeding. If an injury was presumed, the fish was caught with a dip net and examined more intensively. Animals of group F1 were euthanized 2-24 h post exposure, while cod of other groups were sacrificed two weeks after exposure using an overdose of MS-222. Ten minutes following cessation of the opercular movement, death was physically confirmed by cutting the gill arches and bleeding the animals. At necropsy the total length (from tip of snout to end of the tail; L) and the weight of the cod were determined. All fish were examined for external abnormalities with special focus on darkening or blackening of the tail region, external bleedings and wounds. The weight of the gonads, intestinal tract, left epaxial muscle (Wm) and somatic weight (eviscerated fish; Ws) were recorded. The weight of the gonads was used to calculate the Gonado Somatic Index (GSI) (Lambert and Dutil, 1997b). The Intestine Somatic Index (ISI) was determined by dividing the intestinal tract weight by the Ws. The Ws was used to calculate the condition factor of the animal, expressed as Fulton's condition factor (K), with formula $K= 100*Ws\ L^{-3}$ (Bagenal, 1978). In addition, the muscle factor (M) was calculated with the formula $M= 100*Wm\ L^{-3}$.

Internal organs were also examined for lesions and samples of gills, dorsal muscle (base of 3th dorsal fin), heart, liver, spleen, gut and kidney were collected and processed for histological examination and compared with the control animals as described by Soetaert *et al.* (2015b). After the autopsy, fish carcasses were labelled and frozen, and lateral and dorso-ventral X-rays (60kV, 12.5 mAs) were taken. For the F2 and F3 animals, radiographs were taken at the Nofima infrastructure in Tromsø (Siemens Nanodor 2 X-ray machine), while all other radiographs were taken at Ghent University (EDR6 CANON, type CXDI-50G, flat panel detector, scintillator and amorphous silicon Sensor LANMIT 4, Santa Clara, California, USA). All photographs were examined to detect possible malformations, fractures or luxations. Additionally, the number of vertebrae was determined for minimum ten individuals per group.

The 22-25th vertebral body (predilection region for spinal injuries) of 15, 10, 6, 6 and 6 fish of group W, F1, F2a, F2b and F3 was collected, respectively. These vertebrae were boiled, brushed in running tap water, immersed for four days in 100% acetone for dehydradation and removal of fat, and dried at room temperature. Thereafter, the 23rd vertebra was detached from the others, dried at 65°C to constant dry weight, and weighed with a precision of 10 µg (Mettler-Toledo, XP 205). The dried 23rd vertebrae were then incinerated for 7 h at 850 °C and the ashes weighed (=mineral weight) (Sbaihi *et al.*, 2007). The mineral ratio (MR) was then calculated as follows: MR (%) = mineral weight dry weight$^{-1}$.

## Statistical analysis

To cope with the small sample sizes and high amount of zero's (no effect) in the data set, Fisher Exact Tests were performed to compare treatments in which at least one effect was encountered. The data were pooled to analyze the effect of 4 variables: (i) a difference in response after identical exposure (I) between wild and farmed fish exposed, (ii) a difference in response after identical exposure (I) between farmed F1 fish and farmed F2 and F3 fish, (iii) a difference in response between F1 fish exposed to 60 or 120 V and (iv) a difference in response between F1 fish exposed to 120 V with and without inductor.

# Results

## Behavioural reactions

The **behavioural reaction during exposure** was similar for all cod, irrespective of the exposure, in that they showed a cramp reaction until the end of the electrical stimulus. During the cramp reaction, the fish's body remained straight and no horizontal nor vertical bending was observed, whereas the head pushed backwards and the opercula were distended. According to the fish manipulator's observation, animals exposed against the Delmeco electrode showed stronger contraction than those exposed to the thinner Marelec electrodes (pulse IDs IX & X), resulting in a faster and more powerful displacement of the stick that was used to fixate the fish during exposure. In search of an explanation, a simulation of the electric field strength around and in the cod was made and presented in Figure 6.2. In the direct surrounding of the thin Marelec electrodes the field strength is higher but it also shows faster exponential decay. Consequently, field strengths inside the fish's body are smaller.

The **behavioural reaction immediately after exposure** at 60 V was variable. No reaction was observed in 10-20% of the cod, 60-70% showed weak escape behaviour and swam away quietly and 10-30% of the fish showed very agitated swimming and occasionally jumped out of the open triangular cage. In general, fish that had shown a more agitated behaviour prior to exposure, exhibited a stronger flight reaction. When the potential difference on the Delmeco electrodes was doubled to 120 V (pulse IDs IV & V), 11 out of 20 animals showed epileptiform seizures, which was a significant increase compared to 0 out of 50 exposed to 60 V ($p<<<0.05$). The highest number of epileptiform seizures was observed in cod exposed to the highest power (Table 6.1, pulse ID IV, 18 kWp) without an inductor. The number of epileptiform seizures was much lower when an inductor was used and the power dropped to 15.6 kWp (Table 6.1, pulse ID V), although this difference was not significant ($P=0.28$).The seizures consisted of myoclonic jerks resulting in a lack of responsiveness during which the fish were lying on their side not showing opercular movement. The latter slowly returned after 30-90 s, with these fish showing uncoordinated behaviour up to 30 minutes following exposure and dazed swimming capabilities during the first few hours post exposure. This was previously reported during homogeneous exposures between plate electrodes (Soetaert *et al.*, 2015b) and when juvenile cod were exposed in a near heterogeneous field set-up (De Haan *et al.*, 2011).

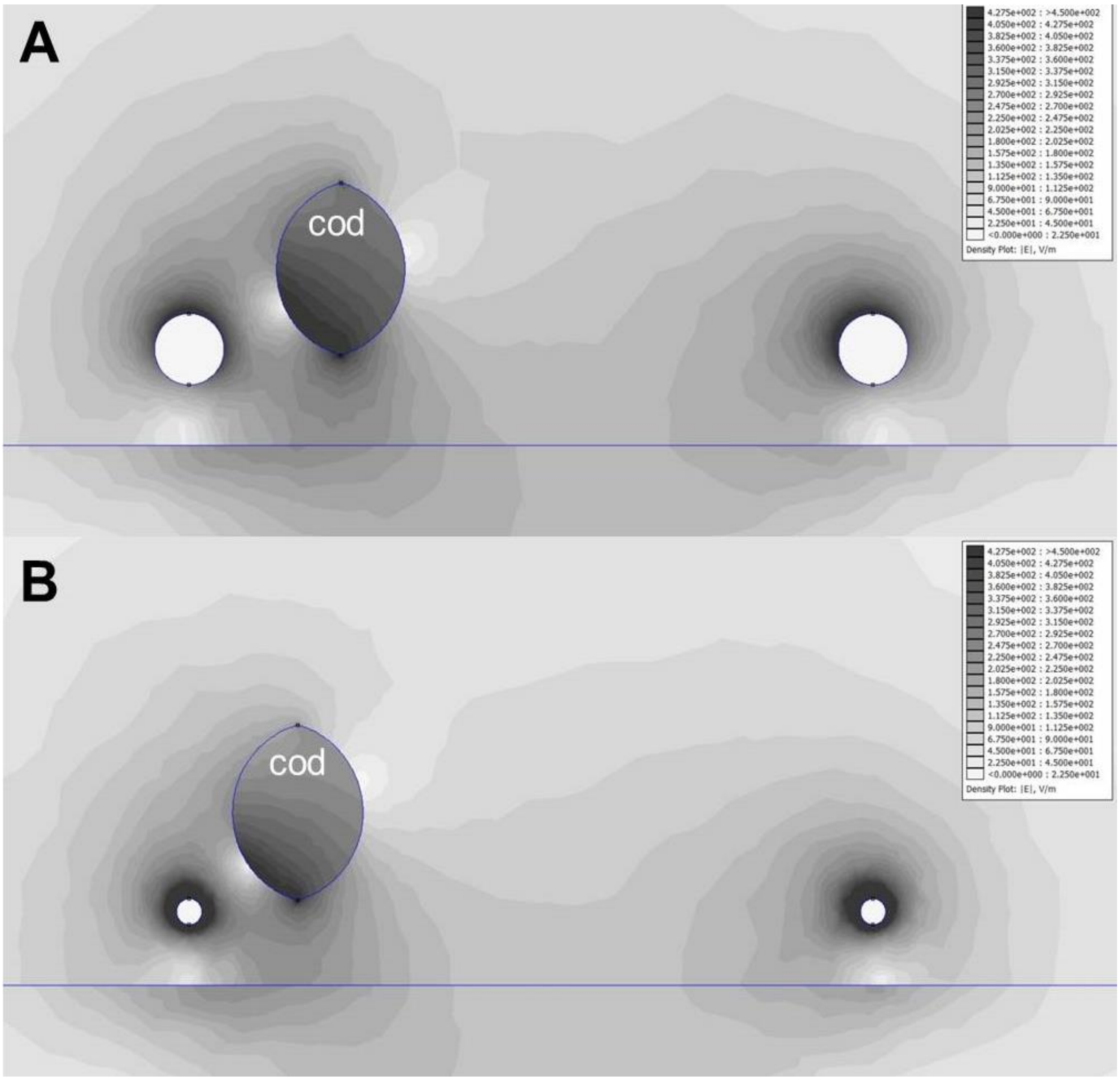


**Figure 6.2:** Qualitative approaching simulation of a cross section of a cod exposed to the Delmeco electrodes (Ø=26 mm, 4A) and Marelec electrodes (Ø= 12 mm, 4B), at 60 V difference with a mutual distance of 0.32 m. Lighter grey representing lower field strengths are present inside cod exposed to the thinner electrodes (B).

## Physical injuries

During autopsy**, paravertebral haemorrhages** were observed in five cod. In four fish (W, pulse ID I; F1, pulse ID II; F1, 2 x pulse ID V) a dark discoloration of the skin located ventral to the second dorsal fin was observed immediately following exposure and in one animal (F1, pulse ID V) blood originating from the anal opening was noted (Table 6.1). Immediately after release in the housing tanks, these animals were able to swim upstraight but showed less active behaviour. When a dip net entered the tank, they joined the other fish. The W cod displaying the darker skin coloration swam more near the water surface compared to other cod during the follow-up period, but they showed normal escape behaviour in case a dip-net appeared. No abnormal opercular movement nor loss of equilibrium was observed.

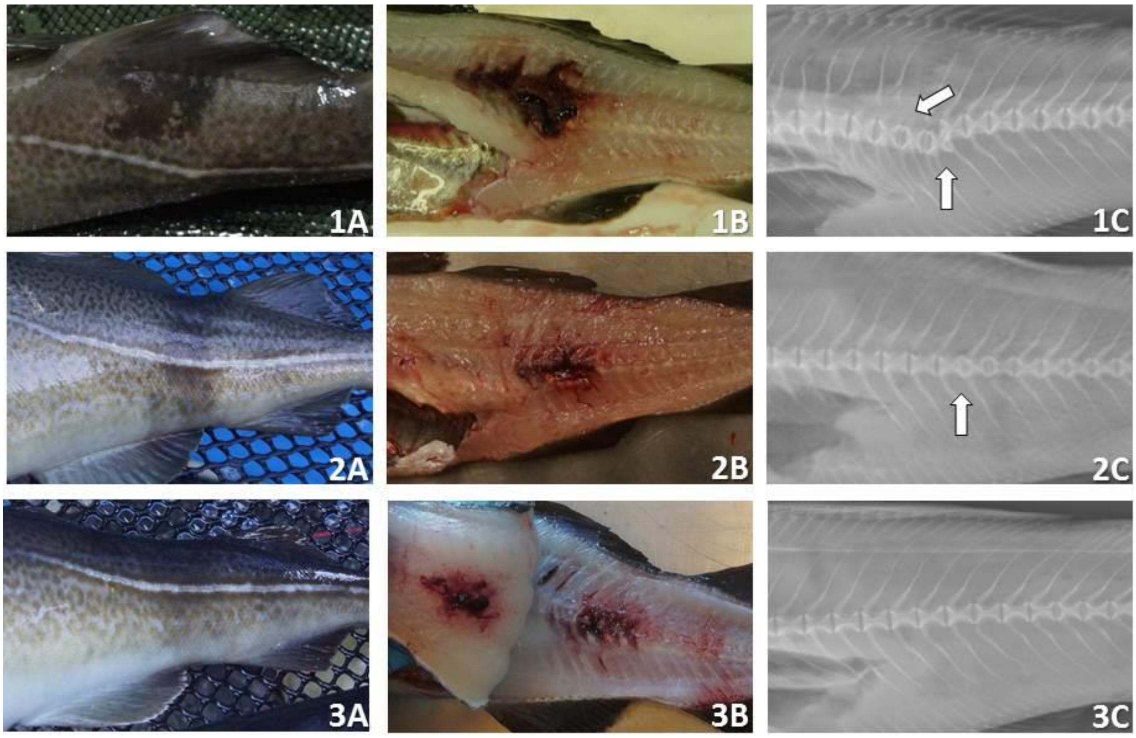


**Figure 6.3**: Wild cod showing spinal luxation (1), farmed F1 cod with spinal subluxation (2) and affected F1 cod without spinal injury (3). Figures ‘A’ show the dark discoloration immediately after exposure, figures ‘B’ display the paravertebral haemorrhages noted 14 days (1B) or 2 h (2B & 3B) post exposure, and figures ‘C’ depict X-rays of an acute luxation with associated bone fractures (1C), an acute subluxation (2C) and an undamaged spine (3C).

**X-ray analysis** disclosed acute spinal injuries in 3 out of 5 fish with paravertebral haemorrhages, located between the 20 and 25th vertebra. This region was the same for all injured cod encountered. The affected W cod showed a complete luxation with associated bone fractures (Figure 6.3, 1c), whereas the two F1 fish had a slight subluxation (Fig.ure 6.3, 2c). No statistic difference in number of injuries or haemorrhages was found between wild and farmed fish (P=0.25), between F1 cod (I+II+III) and F2+F3 cod (pulse ID I) exposed to the same pulse settings (80 Hz, 60V) (P≥0.56), between F1 cod exposed to 60 V (pulse IDs I+II+II) and F1 cod exposed to 120 V (pulse IDs IV+V) (P≥0.07) or to F1 cod exposed to 120 V without inductor (pulse IDs IV) and with conductor (pulse ID V) (P≥0.23). No spinal dislocations were noticed on the X-ray images taken from the other two cod showing paravertebral haemorrhages (Figure. 6.3, 3). Furthermore, X-ray examination revealed chronic malformations of the vertebral column in cod of all groups. This consisted of two adjacent compressed vertebrae in 5% and 20% of cod of groups W and F1, respectively. In approximately 60% of cod of groups F2 and F3, either chronic compression of three or more adjacent vertebrae, ankyliosis, lordosis or chronic dislocation of more than one neural and hemal arch were observed. The mean number of vertebral bodies observed in cod included in this study is presented in Table 6.2.

Necropsy of the animals without spinal injuries revealed no macroscopic external or internal acute lesions. The mean results of the length, total and somatic weight (eviscerated fish; Ws), Gonado Somatic Index (GSI), Intestine Somatic Index (ISI), Fulton's condition factor (K) and muscle factor (M) are presented in Table 6.2. Upon inspection of the gills of F2 cod, multifocal white discolorations were seen in 25% of the animals. When examining the internal organs, nematodes were found in liver of wild cod, while multifocal granulomas were often present in the liver, spleen and kidney. In all other animals, no macroscopic abnormalities were found. **Histological examination** did not reveal any acute lesions. Melanomacrophage aggregates were observed in all spleen and kidney samples and half of liver samples of W cod. These aggregates were present in only 4% of F1 fish and 30% of F2 and F3 fish. They were often located in the kidney, sometimes also in the spleen. Incidental findings included multifocal mild gill hyperplasia with intralesional protozoa in F2 cod, nematodes in liver of wild cod, and multifocal granulomas in the liver, spleen and kidney of wild cod.

**Table 6.2:** Mean (± s.d.) of different physiological parameters of cod.

| Cod of group | W | F1 | F2a | F2b | F3 |
|---|---|---|---|---|---|
| Total length (cm) | 39.0 (±5.4) | 40.1 (±2.2) | 68.6 (±3.8) | 72.5 (±4.1) | 45.28 (±2.5) |
| Weight (kg) | 0.6 (±0.3) | 0.8 (±0.2) | 4.0 (±0.8) | 4.3 (±0.7) | 1.0 (±0.2) |
| Somatic weight (%) | 91.3 (±1.4) | 81.8 (±1.0) | 69.4 (±10.7) | 79.3 (±6.7) | 85.2 (±3.4) |
| Condition K-factor | 0.82 (±0.09) | 1.04 (±0.07) | 0.85 (±0.11) | 0.90 (±0.13) | 0.94 (±0.08) |
| Muscle M-factor | 0.34 (±0.06) | 0.61 (±0.06) | 0.35 (±0.05) | 0.43 (±0.08) | 0.48 (±0.06) |
| GSI (%) | 0.4 (±0.2) | 0.5 (±0.4) | 25.0 ±22.8) | 2.2 (±1.9) | 3.2 (±2.1) |
| ISI (%) | 9.1 (±1.6) | 21.3 (±1.5) | 19.7 (±6.0) | 20.6 (±5.4) | 14.2 (±2.6) |
| # vertebrae | 52.6 (±0.7) | 51.8 (±0.7) | 51.5 (±0.5) | 51.5 (±0.5) | 52.5 (±1.0) |
| Vert. min. cont. (%) | 64.3 (±0.9) | 61.8 (±0.3) | 60.7 (±0.6) | 61.0 (±0.2) | 60.4 (±0.7) |

The **mineral ratio** is summarized in Table 6.2. After thawing of samples, the vertebrae of the F1-fish were notably more loosely connected and much easier to disconnect during processing before and after boiling of these vertebrae. In all groups, vertebrae of larger animals also seemed slightly tighter connected than those of small individuals.

# Discussion

## *Observed effects*

All cod showed a **cramp reaction during exposure**. This was expected as the threshold frequency inducing a cramp reaction in vertebrate species is reported to be between 20 and 30 Hz (Snyder, 2003; Soetaert *et al.*, 2015b) which is well below the frequencies applied in the present experiments. However, this cramp reaction was less powerful in cod exposed near the thinner Marelec electrodes (Ø= 12 mm) (pulse IDs IX, X) as compared to the thicker Delmeco electrodes (Ø= 26 mm). This may be explained by larger electrodes having less electric resistance in water, resulting in larger radiated electric fields (Novotny, 1990). If the diameter of the electrode is doubled, the current density at every point external to the electrodes will also be twice as large if the potential difference is applied. This phenomenon is illustrated in the FEMM simulation, demonstrating lower field strengths inside the fish's body part when lying near the thinner electrode. Nevertheless, it is remarkable that this surpasses the impact of only a small part of the cod's body being exposed effectively. Indeed, the 0.18 m long Delmeco electrodes covered only the most cranial part of the 45 cm large fish, whereas the entire body of the cod was exposed near the 1 m long Marelec electrodes. This suggests that the stimulation of receptors located in the cranial part of the fish's body may be more decisive for the intensity of the muscle contraction than direct muscle stimulation.

The **behavioural reaction of cod immediately after exposure** to the cramp pulse used by electrotrawls targeting sole (60 V) was an escape response. However, when the amplitude was doubled to 120 V, and consequently the power almost quadrupled, epileptiform seizures occurred in 11 out of 20 individuals. These epileptiform seizures result from overstimulation of the brain, which explains the correlation with power and field strength. Indeed, field strength is, next to frequency, reported as the predominant factor in generating epileptiform seizures (Sharber and Black, 1999; Roth *et al.*, 2004; Soetaert *et al.*, 2015b). Sharber *et al.* (1994) stated that the myoclonic jerks associated with these epileptiform seizures induce spinal injuries. This was not confirmed by our data since none of the 11 cod showing epileptiform seizures were injured, whereas three of the nine animals remaining fully conscious during exposure displayed paravertebral haemorrhages. Moreover, in previous studies reporting spinal injuries when exposing adult cod to commercial pulse settings up to 60 V, epileptiform seizures were never observed (De Haan *et al.*, 2011).

### Physical injuries

**Acute spinal injuries** are often associated with dark discoloration of the integument which is believed to be caused by dilatation of skin melanophores, possible as a result of sympathic nerve damage and stimulation (Sharber and Black, 1999). In the present study, spinal injuries diagnosed by means of radiographic imaging were always associated with haemorrhages in the musculature flanking the vertebral column. However, no spinal injuries were encountered in two out of the five animals displaying paravertebral haemorrhages. This suggest a temporary dislocation of vertebral bodies had occurred, possibly eliciting a tear in the caudal vessels situated in the hemal canal, where after the vertebrae resumed their normal position. Besides, the predilection site of the spinal injuries was an intriguing finding. Indeed, the latter were always located between the 20 and 25th vertebra, ventral to the caudal part of the second dorsal fin, as also reported previously (van Marlen *et al.*, 2014; De Haan *et al.*, 2011; Soetaert *et al.*, 2015b). This is the transitional zone between the abdominal and tail region, where the compression force imposed by the lateral muscle during swimming is presumed to be the strongest. The latter was also put forward as an explanation for the finding that the 23th vertebral body is also most frequently affected by lordosis in farmed cod (Opstad *et al.*, 2013). In other species, the location of spinal injuries varies (Snyder, 2003), and is believed to occur in those regions with the strongest muscle contractions (Dolan and Miranda, 2004). Remarkably, the part of the body in which the spinal injuries were located, was not situated between the conductors and thus barely electrically stimulated. Hence, it is unlikely that these spinal injuries were caused by direct muscle stimulation or stimulation through the sensory nerves as suggested by McBary (1956). The present data point toward a reflex response after stimulation of the cranial sensory nerves and/or brain. This hypothesis would also explain the weaker contractions of cod exposed with the entire body was exposed near the Marelec electrodes. Indeed, the weaker behavioural reaction to the Marelec electrodes may result from the lower field strengths around the animal's cranial body part, regardless of the much higher field strengths surrounding the caudal part of the body. In addition, direct stimulation of the muscles would probably result in asymmetric cramps as the fish's side nearest to the conductor is stimulated more intense. These elements might suggest that stimulation of the cranial sensory nerves and/or brain is (co-)decisive for the intensity of the noted reactions. More research is needed to endorse this hypothesis.

## *Influence of electric settings and set-up*

The results in Table 6.1 show that the **electrical output of the** laboratory **pulse generator** (pulse ID I) was similar to that of the Delmeco generator (pulse ID II) used in commercial fishing practice and in the study of de Haan *et al.* (2011). The latter research group applied the following pulse settings: 80 Hz PBC, 57Vp, 68 Ap, 254 µs pulse duration, and a 220 µs rise time. In the present study, an identical experimental set-up (identical exposure cage, electrode pair, electrode distance, powerleads) and almost identical pulse parameter settings (80 Hz PBC, 60Vp, 72-82 Ap, 270-310 µs pulse duration, and a 40-220 µs rise time) were used for all cod experiments (pulse IDs I, II, III, VI, VII, VIII) when the commercial settings were simulated. Moreover, the same electrodes, wires and set-up were used. However, spinal injuries were observed in only 0-5% of the fish, which is far less than the 50-

70% observed by de Haan *et al.* (2011). This indicates that the differences in injuries with the latter study do not originate from differences in exposure with the reference treatments (pulse IDs I & II) in the present study. When additionally the pulse amplitude and power was doubled and quadrupled respectively, the injury rate of F1 cod increased to 0-30%. This inverse correlation between the occurrence of spinal injuries and/or paravertebral haemorrhages and pulse amplitude was previously observed by several authors (Snyder, 2003; Schreer *et al.*, 2004; D. de Haan *et al.*, 2011). Nevertheless, this injury rate is still much lower than the 50-70% reported by De Haan *et al.* (2015).

Besides, several **other electric settings** were altered to cover variability taking place in commercial fishing practice. First, a temperature ranging from 3.5°C to 15°C was tested, including the temperature at which De Haan *et al.* (2015) obtained 50-70% injuries, but no marked impact was observed. Second, the Delmeco electrodes were compared with thinner Marelec electrodes. Although the Marelec electrodes are currently only used in electrotrawls targeting shrimp, they have recently been experimentally tested in electrified benthos release panels in combination with a cramp stimulus for sole and also induced ±8% spinal injuries in cod (Chapter 8). This indicates that even the weaker contractions in the proximity of the Marelec electrodes can cause significant injury rates, exceeding those observed in the present study. Third, the differences between treatment with and without inductor (pulse IDs IV vs V) suggest that the rise time of the pulse may affect the behavioural reaction and injury rate, although no significant effect could be evidenced. Consequently, electrotrawls using the HFK-system with short rise time may have a different effect on cod than those using the Delmeco system with longer rise time. Although in our experiments, all spinal injuries encountered in F1 fish exposed to long rise times, the opposite was observed in fresh water electrofishing research where much higher injury and mortality rates were noted upon adopting exponential and quarter-sinus pulse shapes with steep initial slopes (Halsband, 1967; Vibert, 1967; Lamarque, 1967a,b, 1990). Last, the pulse type was switched from PBC to PAC (pulse IDs VI & X) and the orientation of the animal was switched to perpendicular (pulse ID VII) but no increase of injuries or behaviour was observed, although this may result from the low number of animals included and/or the low numbers of injuries observed.

The present and previous data show **variability in the occurrence of electric-induced spinal injuries**. De Haan *et al.* (2011) proved that cod exposed above or further than 15 cm from the electrodes did not show spinal injuries, but that that up to 70% of the animals can be harmed when exposed in the close proximity near the conductor. These findings accord to the 8-11% injury rates observed in the field (van Marlen *et al.*, 2014; Chapter 8), with cod being exposed at random positions relative to the electrodes. However, only 0-5% of the cod exposed under very similar to identical conditions near the electrodes were observed with spinal injuries in this study. Although pulse parameters such as the applied potential difference and electrode diameter could affect this injury rate, they could not elucidate the differences with previous studies. It can therefore be concluded that the large variability in occurrence of spinal injuries is not only determined by the electric pulse parameters but that also intrinsic fish properties may play a crucial role and hence need to be taken into account upon performing studies on the impact of electric fishing.

## *Fish-related parameters*

Since pulse and electrode settings were identical to those applied by de Haan *et al.* (2011) but resulted in very few lesions, **fish-related parameters** may have a much larger influence on the occurrence of spinal injuries in cod than previously assumed. Unfortunately, all cod-groups included showed very low injury rates and as a consequence, no significant differences in sensitivity between the cod could be demonstrated. Nevertheless, important morphological differences are observed between the different groups, which may reveal the first indications of possible decisive parameters and allow a detailed comparison with future experiments.

Cod shows **seasonal dynamics** in cod's condition and muscularity (Schwalme and Chouinard, 1999), which may affect the fish's reaction and muscle contractions induced by electric pulses. Therefore, physiological parameters were monitored based on the muscle factor (M) and the Fulton's condition factor (K). The latter is linearly related to the muscle protein content in wild cod, which acts as a parameter for the muscle quality and the energy reserves of the fish (Lambert and Dutil, 1997a). This condition factor, as well as the somatic weight, show seasonal differences which are distinguishable in the results of the farmed fish: April (F2a: K=0.82), May (F3: K=0.94), June (F2b: K=0.90) to October (F1: K=1.04). Wild cod had a K-factor of 0.82, which is close to the limit of 0.85 as being observed in well fed wild cod (Lambert and Dutil, 1997b). These K-values seem to be proportional with muscularity, expressed as the M-factor, as they were up to 79% higher in farmed cod compared to wild cod (Table 6.2). As mentioned above, muscular contractions are probably the main cause of spinal injuries. Therefore, it is tempting to speculate that an increased muscularity will result in a higher incidence of spinal injuries after electric exposure. However, no difference in injuries (Table 6.1) was observed between the weakened F2a fish with low M-factor and F2b cod with a higher M-factor (Table 6.2). Additionally, F1 fish showing a much stronger muscularity than wild cod, showed only a 3% injury rate, which is much lower than observed in the field by van Marlen *et al.* (2014). Although muscularity can play a role, these findings demonstrate that other parameters need to be included as well.

The **size of the animal** can also be a determining factor. It is generally accepted that larger fish, with a larger potential difference over its body, will react more intensively to electric pulses (Emery, 1984; Dalbey *et al.*, 1996; Dolan and Miranda, 2003). Indeed, in studies of De Haan *et al.* (2011), small cod (12-16 cm) exposed to electric pulses did not develop lesions. Dalbey *et al.* (1996) also found a positive correlation between the number and severity of the injuries and the size of rainbow trout (*Oncorhynchus mykiss* W.), with larger animals showing more fractures and less vertebral compressions after electric exposure. De Haan *et al.* (unpublished data) in contrary observed a decrease in fractures with body size in adult cod, although no injuries were observed in juvenile cod (0.12-0.16m). In the present study, the largest cod of the F2 group did not show fractures. These observations make it tempting to speculate that small animals are also susceptible. This is in line with the results obtained by van Marlen *et al.* (2014) who described paravertebral haemorrhages in wild cod of 0.20-0.27 m. However, as each fish group had a different origin and hence rearing history, other (co)decisive factors in terms of sensitivity to electric pulses cannot be excluded at this stage.

**Rearing conditions** affect the phenotype and morphology of fish. Differences in rearing conditions may not only result in irreversible changes in phenotype (Galloway *et al.,*1998 and 1999; Johnston *et al.*, 1998), they also can influence the number of vertebrae (Blaxter, 1969; Brander, 1979; Lear and Wells, 1984), mineralization (Kousoulaki *et al.*, 2010) and deformities of the spinal column (Fjelldal *et al.*, 2007; Fjelldal *et al.*, 2009; Fjelldal *et al.*, 2013). Important parameters that affect the spinal column are water quality and temperatures as well as feed and nutrition. Since the different groups of cod had different origins, the possible effect of rearing, the muscularity, mineralization and skeletal deformities were determined and compared.

Differences in the **number of vertebrae** and muscle mass was previously suggested as a possible reason for differences in vertebral injuries observed between salmonids and centrarchids in freshwater and cod and seabass in seawater (Soetaert *et al.*, 2015a & c). The number of vertebrae varies between specific natural populations (Swain *et al.*, 2001) but can also be influenced by environmental parameters. Blaxter (1969) stated that water temperatures, salinity and oxygen levels during early life stages may affect the number of vertebrae of teleosts. In addition, Brander (1979) reported an inverse relationship between water temperature and vertebral number during early development in Atlantic cod. Despite their different rearing history, cod of all our experimental groups had 52±2 vertebrae (Table 6.2) and no aberrant numbers were found in the affected cod (Table 6.3). Moreover, similar values are reported by De Haan *et al.*, (unpublished data) and described in literature for cultured and wild cod (Fjelldal *et al.*, 2013). It is therefore unlikely that the number of vertebrae is responsible for the observed variability between both studis.

**Table 6.3:** The mean (±s.d.) of different physiological parameters of cod, both for the unaffected exposed animals and for the individuals that showed paravertebral haemorrhages in different electric settings (I, II, V).

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

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

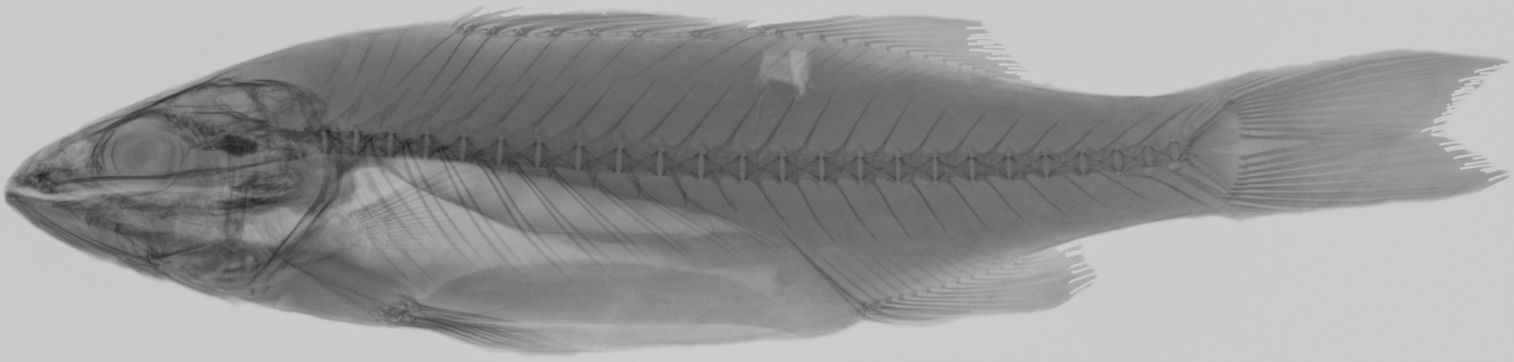
The mechanical strength of the vertebral column is also determined by the strength of the **inter-vertebral ligaments**. A weaker intervertebral connection may directly promote the occurrence of (temporary) luxations. It is therefore important to stress the observation that the vertebrae of the F1 fish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

None of the previously discussed **breeding parameters** revealed a conclusive effect on injuries. The decisive factor(s) explaining the intriguing large difference in spinal injury rates between the cod exposed by De Haan *et al.* (2011)

and the F1 cod in the present study, raised at the same farm and exposed identically at the same time of the year, is (are) yet to be discovered. The rearing history of the animals might set us on the road to elucidate the determining parameter(s) as further inquiry indeed showed differences in rearing circumstances. The larval life stages of the cod used by De Haan *et al.* (2011) were reared extensively in a lagoon of which the temperature reached 20°C in summer, and they were fed zooplankton as described by Blom *et al.* (1991). In contrast, all farmed cod used in the present study were reared intensively in closed tanks and fed with rotifers and *Artemia* (Hansen *et al.* 2014). Both the temperature and the feed are known to have an influence on the fish's development and growing speed (Galloway *et al.*, 1998 and 1999) and on the phenotype of numerous characteristics critical to swimming (Johnston *et al.*, 1998). Faster growing speeds might for example affect intervertebral connections, making fish more prone to luxations. Although our key-question remains unanswered, i.e. which parameter is decisive for the variable number of lesions in cod, the data as obtained in this study offer intriguing additional perspectives for further research.

# Conclusion

The use of thicker electrodes and higher pulse strengths was positively correlated with the intensity of the reaction and the numbers of injured cod observed. Besides, the present results demonstrate that the sensitivity of cod to develop electric-induced spinal injuries can vary between 0 and 70% under (almost) identical exposure near the electrode. This indicates that these injuries are not only determined by the pulse parameter settings, but also by subtle fish-specific parameters. Although the absence of a sensitive group of cod did not enable the elucidation of the decisive parameter, the effect of intervertebral ligaments and rearing conditions during early life merit attention in future research.



# CHAPTER 7

# EFFECT OF ELECTROTRAWLS' ELECTRIC CRAMP PULSE ON EUROPEAN SEA BASS

# Abstract

Pulse trawling is a promising alternative for beam trawling in the brown shrimp and sole fishery. However, lesions, such as spinal injuries can occur in cod and whiting, which could indicate that roundfish are more susceptible to electric pulses. Therefore, we aimed to assess the vulnerability of another roundfish: sea bass (*Dicentrarchus labrax L.*). Sea bass were divided in 2 groups based on the size of the animals and exposed to electric pulses as used in electrotrawls targeting sole. Thereafter, the animals were daily monitored and euthanized 14 days after exposure for gross, radiographic and histologic examination. In none of the animals, lesions were found. This suggests that sea bass is less vulnerable than cod and that other parameters besides anatomy of the musculature have to be taken into account when examining the effect of electric pulses.

# Introduction

Pulse trawling with electrotrawls is a very promising alternative for conventional beam trawling. In these gears, mechanical stimulation by tickler chains or bobbins is partially replaced by electric pulses. The optimal pulse settings to target Dover sole (*Solea solea* L.) comprise a bipolar pulse of 40 to 80 Hz resulting in a cramp reaction (Soetaert *et al.*, 2015a). The removal of the tickler chains combined with the lower towing speeds reduces the drag drastically, resulting in fuel savings up to 50%. Moreover, significant reductions in benthos discards between 16 and 62% are reported (Rasenberg *et al.*, 2013; van Marlen *et al.*, 2014). Although exposure to electric pulses inducing a cramp reaction did not result in severe lesions in sole (Soetaert *et al.*, 2015b) nor in dogfish (*Scyliorhinus canicula L.*) (De Haan *et al.*, 2009a), spinal injuries were elicited in Atlantic cod (*Gadus Morhua L.*) both under field (Van Marlen *et al.*, 2014; Chapter 8) and experimental conditions (De Haan *et al.*, 2011; Soetaert *et al.*, 2015b,c). Also in whiting (*Merlangius merlangus* L.), spinal injuries were noted following exposure to electric pulses (Van Marlen *et al.*, 2014). The above data made us hypothesize that the observed variation in susceptibility between roundfish and flatfish is rooted in their differing morphology more specifically their body musculature. To rectify this hypothesis, the current study aimed to assess the impact of electric pulses on sea bass (*Dicentrarchus labrax* L.), a roundfish species inhabiting the North Sea hence possibly exposed to electric pulses.

# Material & Methods

Fourty-four sea bass were obtained from a commercial farm (Ecloserie Marine de Gravelines, France), acclimatized for 4 months and fed 3 times a week (Marico Supreme 16, Coppens International bv.). They were divided in two groups based on size. Group 1 and 2 consisted of 29 smaller sea bass (31.3 ± 2.2 cm) and 15 larger sea bass (42.1 ± 2.5 cm), respectively. All animals were housed together in the same tank as described by Soetaert *et al.* (2015b&c). The experiments were approved by the Belgian (ID 2011/170) ethical committee.

The sea bass were exposed near wire-shaped commercial electrodes resulting in a heterogenous electric field as described by de Haan *et al.* (2011). The 60 V potential difference over the electrodes was applied by a laboratory pulse generator (LPG, EPLG bvba, Belgium) capable of reaching a maximum output of 150 V, 280 A and 42 kW. The generator was also equipped with a feedback system to ensure that the output exactly matched the set values. This was again controlled using an oscilloscope (Tektronix TDS 1001B). Pulse settings similar to those applied by commercial electrotrawls targeting sole were used: frequency = 80 Hz, pulse duration = 0.25 ms, duty cycle = 2%, pulse type = pulsed bipolar current, exposure length = 2 s.

All animals were individually transferred to an exposure tank as described previously (Soetaert *et al.*, 2015b&c). Twenty and 11 animals of group 1 and 2 were exposed to electric pulses, respectively. The remaining animals were not exposed and served as controls. Briefly, the animals were released in a PVC net near the electrode. As soon as the fish was well oriented, it was gently pushed down to fixate it near the electrode. Once the fish could not slip and it was properly positioned parallel against the electrodes, it was exposed to the electric stimulus. When a cramp reaction occurred, the animal was no longer forced in its initial position to allow movement. The animals remained in the netting material for 15 s, where after they were tagged with floy tags in the first dorsal fin to allow individual identification and transferred to their housing tanks. The animals were daily monitored, fed 3 times a week and sacrificed two weeks later, examined for external and internal lesions and their length, total weight and somatic weight (eviscerated fish, Ws) was determined. The Ws was used to calculate the condition factor of the animal, expressed as Fulton's condition factor (K), with formula K= 100*Ws $L^{-3}$ (Bagenal, 1978). Additionally, radiographic and histological examination was performed as described by Soetaert *et al.* (2015c).

# Results

When transferred to the exposure tank, fish showed a slow swimming behaviour in the netting material, mostly pressing their nose in the ends of the net. Immediately after initiation of the electric pulses, all sea bass showed a cramp reaction (Table 7.1). In all but one animals of each group, this was accompanied by distended opercula during the entire exposure time. No bending of the body was observed. The first seconds following exposure, all sea bass showed an escape reaction and swam away from the point of exposure. This reaction varied between a short 2 s swimming behaviour at moderate speed to a more intense 5 s of agitatedly swimming and sometimes trying to jump out of the netting material. No difference in behaviour between the fish of the 2 groups was observed. The control fish showed the same behaviour prior to fixation, but they did not show an escape reaction after being fixated. When released in the housing tanks, all fish returned to their normal swimming behaviour.

**Table 7.1:** Results (mean ± s.d.) of different physiological parameters at post-mortem examination of small and large sea bass.

| | Small | | Large | |
|---|---|---|---|---|
| | Control | Exposed | Control | Exposed |
| Number | 9 | 20 | 4 | 11 |
| Size (cm) | 31.1 ± 2.1 | 31.4 ± 2.2 | 39.5 ± 2.9 | 43.0 ± 1.5 |
| Weight (g) | 331.4 ± 57.9 | 337.7 ± 72.6 | 718.1 ± 180.1 | 905.1 ± 105.0 |
| Somatic weight (g) | 304.4 ± 53.8 | 306.3 ± 72.6 | 641.2 ± 164.0 | 808.1 ± 91.0 |
| Fultons K-factor | 1.00 ± 0.05 | 0.98 ± 0.10 | 1.02 ± 0.06 | 1.02 ± 0.11 |

During the 2 weeks' observation period, none of the animals died and all demonstrated a normal feeding behaviour. At necropsy, no external or internal abnormalities were found. All sea bass had a filled stomach after being fed 24h prior to sacrifice. The mean results of the length, total and somatic weight (eviscerated fish) are presented in Table 7.1. X-ray analysis did not reveal any spinal injuries or acute lesions, but in 15% of the fish, fused vertebrae were observed. The small and large sea bass counted 24.6 ± 0.9 and 24.8 ± 0.4 vertebrae respectively. Finally, histological examination did not reveal any abnormalities in the gills and internal organs.

# Discussion

The initial aim of this study was to investigate the effect of electric pulses on sea bass and aid in the elucidation as to why there is an apparent difference in susceptibility in between fish species. The exposed animals demonstrated a cramp reaction upon electric stimulation, followed by an escape response similar as to what was reported for cod (De Haan *et al.*, 2011; Soetaert *et al.*, 2015b&c), marine flatfish such as lemon sole and plaice (Stewart, 1977; Soetaert *et al.*, 2015b) and invertebrates such as brown shrimp (Soetaert *et al.*, 2014; Soetaert *et al.*, 2015d). However, this did not result in mortality or gross lesions. This is in agreement with the findings of D'Agaro & Stravisi (2009), where no external lesions nor spinal injuries were observed, albeit following employment of completely different pulse settings and electrodes. In the present study, the gills and internal organs were also examined histopathologically to exclude micro lesions. We may therefore conclude that exposure to electric pulses as investigated in the present study did not elicit lesions as investigated 14 d post exposure.

The absence of continuous abnormal behaviour following exposure and both macroscopic and microscopic lesions suggests that sea bass most likely are less susceptible to the possible negative effects of electric pulses than gadiform roundfish such as Atlantic cod and whiting. This may be explained by morphological differences other than musculature anatomy. First, although all exposed fish clearly demonstrated a cramp reaction, indicating that the electric field was able to penetrate the fish's body, the thick ctenoid scales of sea bass may shield the electric field more efficiently than the thin cycloid scales of cod, resulting in lower penetration into the fish's body and reduced effects. Secondly, differences in number of vertebrae were suggested by Soetaert *et al.* (2015a) as a possible reason for differences in vertebral injuries observed in salmonids and centrarchids. The lower number and the subsequent different morphology of vertebrae (± 25 vertebrae) of sea bass contrast to that of gadoid roundfish such as Atlantic cod (± 52 vertebrae), whiting (± 52 vertebrae) and Pollock (*Pollachius virens* L.) (± 53 vertebrae) and also herring (± 53 vertebrae). All these roundfish have high number of small vertebrae and are reported to suffer spinal injuries after electric exposure in trawls or during stunning (Nordgreen *et al.*, 2008; Roth *et al.*, 2004; van Marlen *et al.*, 2014). This suggest that the much lower number of vertebrae may elicit an increased mechanical strength of the sea bass's vertebral column making this animal less prone for the development of spinal injuries.

Interestingly, in freshwater species, Salmonidae (having a similar morphology as cod and ±58 vertebrae, Fraser *et al.*, 2015) were reported to be much more susceptible for spinal injuries than other roundfish such as bass (having ± 31 vertebrae, Jayne and Lauder, 1995) (Zeigenfuss, 1995). Moreover, the high variability in the occurrence of injuries in cod (Soetaert *et al.*, 2015c) was also observed in Salmonidae (Snyder, 2003a), which may suggest that similar morphological parameters may be crucial. However, extrapolation and comparison of different studies should be done with great care. Indeed, there is a high variability between the experimental set-ups of studies but also the animals themselves may play a major role in terms of origin and rearing history (Snyder *et al.*, 2003a; Soetaert *et al.*, 2015c).

In conclusion, the present study did not demonstrate (spinal) injuries in sea bass resulting from electric pulses, indicating differences in sensitivity with gadoid roundfish such as cod and whiting. Therefore, in addition to fish musculature anatomy, other fish parameters, such as the vertebrae, need to be considered when evaluating the effect of electric pulses as well.

# CHAPTER 8

# REDUCING BYCATCH IN BEAM TRAWLS AND ELECTROTRAWLS WITH (ELECTRIFIED) BENTHOS RELEASE PANELS

Adapted from:

Soetaert, M., Lenoir, H. and Verschueren, B. 2015. Reducing bycatch in beam trawls and electrotrawls with (electrified) benthos release panels.
Submitted to ICES Journal of Marine Science, December 2015.

# Abstract

A benthos release panel (BRP) is known for its capacity to release large amounts of unwanted benthos and debris which decreases the impact on these animals and facilitates the on board sorting process. It also reduces the bycatch of undersized fish, which is desired once the European discard ban is implemented. However, unacceptable commercial losses of sole and damage to the BRP as a consequence of suboptimal and unsuitable rigging in the traditional beam trawl with chain mat, hampers a successful introduction in commercial beam trawl fisheries. To eliminate these drawbacks, square meshed BRPs with different mesh sizes (150 mm, 200 mm and 240 mm) were rigged in a trawl with alternative design and tested for selectivity. In addition to this, the effect of electric stimulation at the height of the BRP to eliminate the loss of commercial sole was examined. According to our observations, no abrasion of the net attributable to suboptimal rigging occurred in any of the BRPs tested. The catch comparisons showed significant release of benthos and undersized fish in all panel mesh sizes, but a significant loss of marketable sole in the 150 and 240 mm BRP was still ascertained. Adding an 80 Hz electric cramp stimulus to the BRP, resulted in equal catches of sole larger than 25 cm, without negatively affecting the release of benthos and most undersized commercial fish. Although this study clearly demonstrates the promising potential of electrified BRPs (eBRPs), further optimization by using smaller BRP mesh sizes or optimized electric stimuli is warranted to retain all marketable sole.

# Introduction

Towed demersal fishing gears are used worldwide to extract marine resources. In most cases, optimal use of such gears requires direct physical contact with the seabed to ensure adequate capture rates of target species that live close to, on or within the seabed (Depestele *et al.*, 2015). Demersal fishing techniques affect the marine environment and particularly the benthic communities, reducing their biomass, production and diversity (Lindeboom & De Groot, 1998; Kaiser *et al.*, 2006). Beam trawl impact may result from the direct mortality caused by the trawl and the indirect effects of this mortality on species interactions (Ramsay *et al.*, 1997; Jennings *et al.*, 2002). Fishing with beam trawls causes direct unwanted mortality in invertebrates in two ways. First, injury and mortality is inflicted by physical contact with the shoes, tickler chains or chain mat (Bergman & van Santbrink, 2000). Second, animals caught in the trawl may die from injuries sustained in the net, during hauling, catch sorting or discarding (Lindeboom & de Groot, 1998).

A possibility of reducing the adverse effects of trawling on benthic communities is to reduce the direct mortality by developing alternative fishing methods and through technical modifications. Drop out openings without netting and large diamond and square escape zones just behind the groundrope proved not to be effective in releasing by-catch and induced unacceptable losses of commercial catch (Fonteyne & Polet, 2002; van Marlen *et al.*, 2005). Square mesh windows inserted in the lower panel just in front of the cod end, also known as benthos release panels (BRPs), gave much better results. Field trials with BRPs showed reductions in bycatch of invertebrates up to 80% and reductions in debris exceeding 50% (Fonteyne & Polet, 2002; Revill & Jennings, 2005). These mesh panels may help to release benthic invertebrates immediately after capture, eliminating prolonged retention and compression in the codend. Consequently exposure to the effects of hauling, deck sorting and discarding is avoided, contributing to a better survival of accidentally caught benthic invertebrates (Depestele *et al.*, 2014). Besides, Fonteyne & Polet (2002) report substantial lower bycatches of undersized commercial fish. The latter may increasingly gain importance in the coming years, following the implementation of the discard ban in European fisheries in 2016 because it is expected that the discarding of undersized commercial fish will negatively affect total allowable catches (TAC) (NSAC, 2014). In the past, two main drawbacks have hampered the implementation of the BRP gear modification. First and foremost, the catch of Dover sole (*Solea solea* L.) which is the most important species in terms of economic gain, appears to be adversely affected with unacceptable catch losses of 20-45% (Fonteyne & Polet, 2002; van Marlen *et al.*, 2005). Secondly, the implementation of a rectangular square mesh panel in a traditional beam trawl with a round footrope proved to be suboptimal. BRP rigging resulted in slack and subsequent bag formation in the lower panel, just in front of the BRP, causing abrasion of the net fabric (Personal communication Hans Polet, ILVO, Belgium).

The aim of this study is to tackle these problems. Firstly, it is examined if the release capacity of a BRP inserted in an electrotrawl design with a straight ground rope and a square chain mat improves the release capacity. This is expected because it enables a persisting, stretched geometry of the BRP in the lower panel of the trawl extension. This so called ‘square’ net design is typically used in the pulse fishery, facilitating the lengthwise rigging of the electrodes in the electrotrawl. Secondly, the use of electric stimulation to improve post-catch selectivity was evaluated. So far, research has not demonstrated major side-effects as direct consequence of exposure to electric pulses except for Atlantic cod (*Gadus morhua* L.) and whiting (*Merlangius merlangus* L.) (Soetaert *et al.*, 2015a). Four out of 45 cod exposed caught by electrotrawls were reported to have paravertebral haemorrhages (van Marlen *et al.*, 2014). This was confirmed in laboratory experiments showing 0-70% spinal injuries in cod exposed near the electrodes

(Soetaert *et al.*, 2015b&c; de Haan *et al.*, 2015). However, no major side-effects have been reported in non-gadoid species so far. No increased impact of electric stimulation compared to conventional mechanical stimulation could be evidenced in invertebrate species (Smaal and Brummelhuis, 2005; van Marlen *et al.*, 2009; Soetaert *et al.*, 2014; 2015d). Furthermore, elaborate experiments did not demonstrate lesions or an effect on the 14 day survival of sole, but proved that electric stimuli can elicit a cramp reaction in the fish's muscles, which immobilizes sole (Soetaert *et al.*, 2015b). The second research hypothesis was therefore if adding an electric cramp stimulus to the BRP would prevent marketable sole from escaping through the panel.

# Materials & Methods

## *Rigging of the (e)BRP*

In the present study, the trawls had a straight bobbin- and footrope, as used by electrotrawls, and consequently a rectangular chain mat, resulting in different trawl geometry (Figure 1). Both the standard and the experimental net used in the comparative fishing experiments were constructed in 120 mm polyethylene (PE) netting. In order to reduce wear, the double braided lower panel was provided with bottom chafers made of PE ropes. In the double braided PE codends with nominal mesh opening of 80 mm, an inner PE cod-end with a nominal mesh opening of 60 mm was inserted. The square meshed (e)BRP measured 1.20 x 1.80 m and was inserted in the lower panel of the trawl extension, 10 meshes (1.20 m) in front of the codend of a 4 m flatfish beam trawl equipped with a chain mat (Figure 1), as described by Fonteyne & Polet (2002) and Revill & Jennings (2005). The BRP was made of single braided PE square meshes of three different sizes: 150 mm, 200 mm, 240 mm. Small amounts of chafers were provided every 3th mesh of the BRP to protect it from possible wear without obstructing the BRP outlet. The different nets as well as the BRPs were checked for damage during and after the experimental hauls, and their mesh size was measured using an omega mesh gauge. The difference in mean mesh size of the standard and the experimental codend never exceeded 0.4 mm. Electric power supply to generate the electric pulses on the eBRP was supplied by an on-board winch (EPLG, Belgium) equipped with 250 m reinforced coaxial conductor cable. This cable was attached to the bridle with a strain relief and then lead to a compact pulse generator device (EPLG, Belgium). The pulse generator was attached to the selvedges in the aft of the portside net close to the BRP, firing two electrode wires, each splitting up in three parallel conductors. Small wire shaped conductors (1.2 m, ø 12 mm) were as used and existed of 6 stainless steel strands around a cupper core. These conductors were attached transversally to the 240 mm BRP on a mutual distance of 0.48 m (4 meshes in the BRP) starting from 0.6 m in front of the BRP to the very end of the panel (Figure 1). An electric stimulus as used in commercial electrotrawls targeting sole was applied (80 Hz bipolar pulsed current, 250 µs pulse duration) (Soetaert *et al.* 2015a), aiming for an immobilizing cramp reaction in the sole's muscle to prevent it from escaping through the panel. However the electric potential difference on the electrodes was only 40 V due to power limitations of the generator, whereas ±55 V is used in the commercial trawls (Soetaert *et al.*, 2015a).

The comparative fishing trials were carried out on board R.V. BELGICA (50.9 m L.O.A., 1154 kW) and R.V. Simon Stevin (36 m L.O.A., 2x520 kW). In contrast with commercial beam trawlers, these vessels were not equipped with derrick booms for towing two separate beam trawls simultaneously. To enable comparative fishing, two 4 m beam nets were attached next to each other to an 8 m twin beam trawl with an extra trawl head in the middle (Figure 1), with the (e)BRP always on port side. An overview of the sea trials and valid hauls is given in Table 8.1. Five control hauls before, five hauls during and three hauls at the end of the experiments were performed without a BRP to exclude possible side-specific effects of the beam and trawls. The average haul duration was approximately 1.5 h, covering roughly 45 000 m$^2$ with each trawl.

## *Catch analysis*

The entire catches of both the standard and the experimental trawl were collected in baskets immediately after hauling and the total catch weight was recorded. All commercial fish species were sorted, counted and measured to the centimeter below. All cod were fileted and examined for potential spinal injuries, induced by the electric pulses. The remaining of the catch was separated into a benthos fraction (non-commercial fish & invertebrate species) and a coarse debris fraction (stones, litter and other inert material). These fractions were weighed separately to determine the overall benthos and debris released through the BRP. Subsequently, a subsample (5-8 kg) of the benthos fraction was sorted by species. All animals were counted and the total weight per species was determined. Residual debris in the benthos sample was weighed separately, scaled up and added to the total debris weight. Paired haul catch data, i.e. overall benthos and debris weights on the one hand and numbers of fish and benthos per species on the other hand, were tested on normality and homogeneity based on the Shapiro-Wilk W-test and the Levene's test respectively. If both conditions were fulfilled, a paired sample t-test was used, otherwise the data was compared using a Wilcoxon signed-rank test for paired samples. Concerning commercial fish species, the bycatch reduction was split up into the reduction of undersized and marketable fish. These numbers, as well as these of the benthos species were statistically compared, only if the numbers were sufficiently large (i.e. at least 5 individuals per haul). In this manuscript, all shown numbers include only those animals caught in hauls that accord to these restrictions. The (bycatch) reductions were calculated as the percentage difference on pooled weights based as follows: ((catch experimental trawl – catch standard trawl)/catch standard trawl) * 100. The sampling occurred within ongoing sampling campaigns.

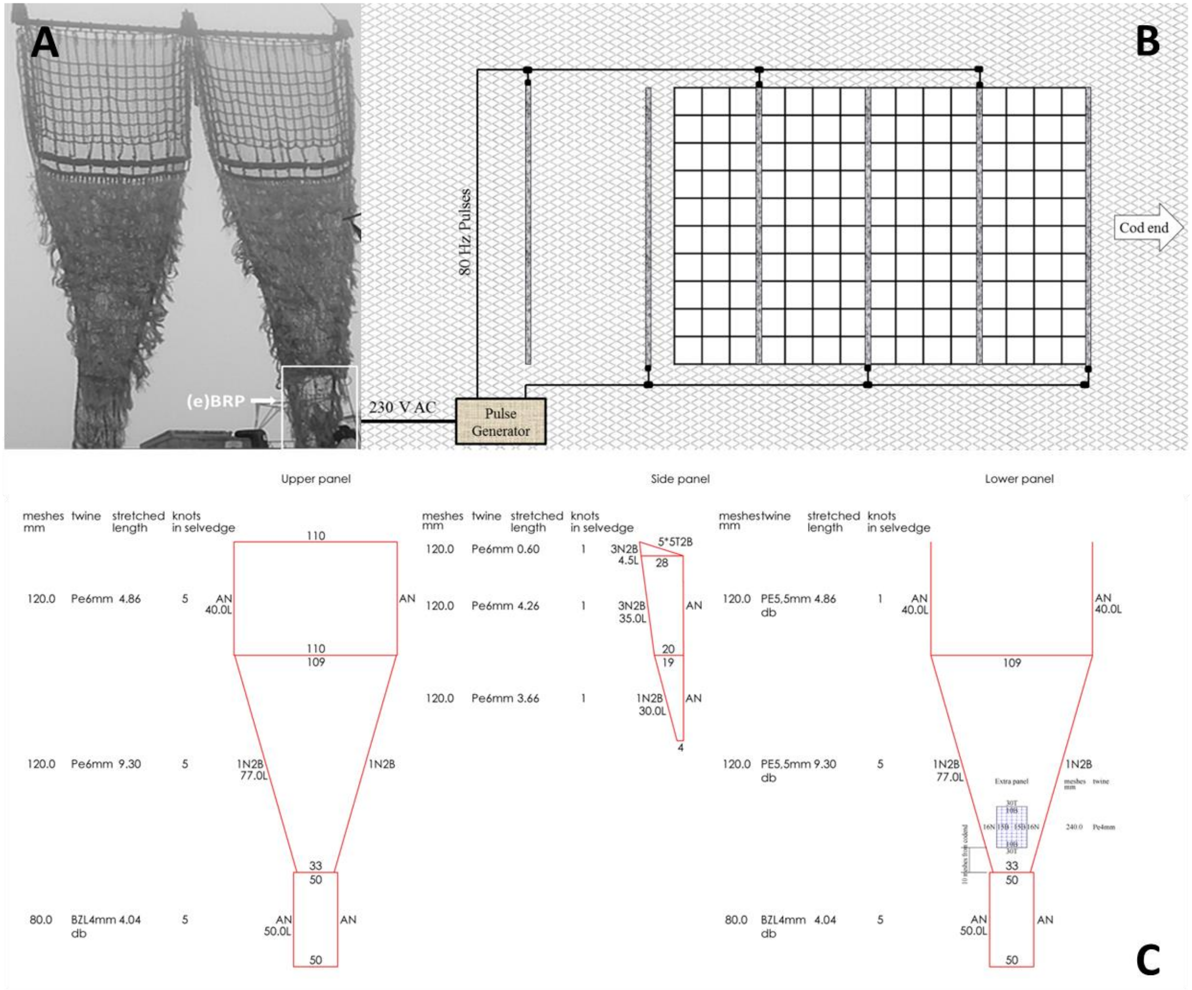


**Figure 8.2:** Rigging of an 8 m twin beam trawl with a reference and experimental net rigged with an (e)BRP (1.20 x 1.80 m) (A), the 240 mm eBRP configuration (B) and the net drawing of the square nets with straight bobbin rope and rectangular chain matrices used (C).

**Table 8.1:** Overview of the sea trials.

| Panel design | Date | ICES area | Location | Vessel | Time | Depth (m) | # valid hauls | Exp. net | Electrodes | Pulse |
|---|---|---|---|---|---|---|---|---|---|---|
| 150 mm BRP | April 2014 | IVc | Shipwash, England | R.V. Belgica | day | 24 - 32 | 10 | starboard | no | no |
| 200 mm BRP | November 2014 | Ivc | Coast, Belgium | R.V. S. Stevin | day & night | 10 - 35 | 5 | starboard | yes | no |
|  | December 2014 | Ivc | East coast, England | R.V. Belgica | night | 24 - 48 | 5 | port side | no | no |
|  | April 2014 | IVc | Shipwash, England | R.V. Belgica | day | 24 - 31 | 2 | starboard | yes | no |
| 240 mm BRP | December 2014 | VIId | NE coast England | R.V. Belgica | night | 30 - 63 | 10 | starboard | yes | no |
|  | December 2014 | IVc | Shipwash, England | R.V. Belgica | night | 24 - 30 | 12 | starboard | yes | no |
|  | February 2015 | IVc | SE coast England | R.V. Belgica | day | 27 - 34 | 1 | starboard | yes | no |
| 240 mm eBRP | February 2015 | IVc | SE coast England | R.V. Belgica | day | 26 - 55 | 16 | starboard | yes | yes |

# Results

## *Control hauls*

Statistical comparison of the 13 control hauls demonstrated no difference between starboard and portside catches in total catch weight (P=0.530), benthos weight (P=0.091) and debris weight (P=0.601). Nor was any significant difference in catch rate observed in the number of sole, plaice (*Pleuronectes platessa* L.), brill (*Scophthalmus rhombus* L.), turbot (*Scophthalmus maximus* L.), dab (*Limanda limanda* L.), lemon sole (*Microstomus Kitt* W.), mullet (*Mullus surmuletus* L.), pouting (*Trisopterus luscus* L.), whiting (*Merlangius merlangus* L.), cod (*Gadus morhua* L.), dogfish (*Scyliorhinus canicula* L.) and ray spp. (*Raya* spp.).

## *Experimental hauls*

The actual inner mesh size of the 150, 200 and 240 mm BRP was 158 ± 3 mm; 178 ± 3 mm and 223 ± 10 mm respectively. The 200 and 240 mm BRP were always nicely stretched in the belly of the net. The 150 mm BRP on contrary was hand-knitted on board, which resulted in a mesh size slightly larger than intended and subsequently a panel length larger than the opening cut out in the lower panel. As a consequence limited, but non progressive, bag formation occurred as a result of the slack. None of the panels showed wear at the end of the experiment and although the chafers were tattered over the end 5-10 cm, no abrasion was seen yet. Besides, no damage was observed to the belly surrounding the BRP or the electrodes.

The total benthos weight was significantly lower for all (e)BRP designs tested (Table 8.2) and all, except the 150 mm BRP showed a significant loss of debris. When analyzed at species level, sea mouse (*Aphrodita aculeate* L.), starfish (*Asterias rubens* L.), whelk (*Buccinum undatum* L.), swimming crab (*Liocarcinus holsatus* F.) and hermit crab (Paguridae) were significantly less abundant in the catches of the trawl fitted with BRPs (Table 8.3). Similar to this, the loss of sea urchins (Echinoidea), blue-leg swimming crab (*Liocarcinus depurator* L.), long legged spider crab (*Macropodia rostrate* L.) and brittlestar (*Ophiura texturata* L.) was (nearly) significant for all (e)BRPs tested. Finally, in line with previous, also the number of non-commercial fish species (Table 8.3) were almost invariably lower in the experimental net fitted with a BRP, although this was only significant for the number of Mediterranean scaldfish (*Arnoglossus laterna* W.) in the 200 mm BRP.

**Table 8.2:** The number of hauls (H), the average catch weights of benthos and debris fractions (± s.d.) for both the standard trawl (A) and the trawl fitted with an (e)BRP (B), the percentage difference in catch of the experimental net compared to the standard net (C) and the p-values (D) of the Wilcoxon signed-rank (w) or paired sample t-test (t) with significant values in bold italics.

| | H | Average benthos weight | | | | Average debris weight | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | A (kg) | B (kg) | C | *D* | A (kg) | B (kg) | C | *D* |
| 150 mm BRP | 10 | 133 ± 49 | 43 ± 18 | -67% | ***0,002*** (w) | 39 ± 38 | 41 ± 46 | 5% | 0,764 (t) |
| 200 mm BRP | 12 | 54 ± 28 | 23 ± 12 | -58% | ***0,000*** (w) | 22 ± 19 | 15 ± 15 | -30% | ***0,010*** (t) |
| 240 mm BRP | 25 | 126 ± 89 | 36 ± 50 | -71% | ***0,000*** (w) | 43 ± 50 | 17 ± 15 | -61% | ***0,000*** (w) |
| 240 mm eBRP | 16 | 119 ± 71 | 22 ± 29 | -82% | ***0,000*** (w) | 18 ± 17 | 5 ± 4 | -74% | ***0,002*** (w) |

The catch data for commercial fish (Table 8.4) shows that sole is lost in all BRP configurations, and significantly in the 150 and 240 mm BRPs. The 41% sole loss in the 240 mm BRP is reduced to 17% when the electric stimulus is added to the BRP. Plaice is caught significantly less in the 240 mm BRP, while this reduction is not observed in the 200 mm BRP or the 240 mm eBRP. The number of surmullet and gurnards (*Chelidonichtys* spp.) is consistently lower if a BRP is used, although this reduction was only significant for the 240 mm BRP. Finally, also dogfish is lost in all BRP set-ups, although only significantly in the 150 and 240 mm BRP.

**Table 8.3:** Total numbers of non-commercial fish species and benthos caught in the standard net (A) and experimental net with BRP (B), the percentage difference in catch of the experimental net compared to the standard net (C) and the *p*-value (D) of the Wilcoxon signed-rank (w) or paired sample t-test (t) with significant values in bold italics.

| | 150 mm BRP | | | | 200 mm BRP | | | | 240 mm BRP | | | | 240 mm eBRP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| **Non-commercial fish species** | | | | | | | | | | | | | | | | |
| *Arnoglossus laterna* | | | | | 357 | 143 | -60% | **_0.010_ (t)** | | | | | 115 | 78 | -32% | 0.284 (t) |
| *Agonus cataphractus* | | | | | 24 | 29 | 21% | 0.742 (t) | 218 | 202 | -7% | 0.842 (t) | 35 | 19 | -46% | 0.500 (w) |
| *Callyonimus lyra* | | | | | 324 | 192 | -41% | 0.328 (t) | 902 | 366 | -59% | 0.305 (t) | 326 | 265 | -19% | 0.685 (t) |
| *Echiichthys vipera* | 59 | 27 | -54% | 0.238 (t) | 315 | 150 | -52% | 0.312 (t) | | | | | 63 | 42 | -33% | 0.324 (t) |
| **Benthic invert. species** | | | | | | | | | | | | | | | | |
| *aequipecten opercularis* | | | | | | | | | 11033 | 2433 | -78% | ***0.000*** (w) | 100 | 0 | -100% | 0.168 (t) |
| *Anemone* (indet.) | | | | | | | | | 290 | 126 | -57% | ***0.030*** (w) | 174 | 13 | -93% | 0.057 (t) |
| *Aphrodita aculeata* | 1745 | 425 | -76% | ***0.020*** (w) | 121 | 30 | -75% | ***0.002*** (t) | 629 | 126 | -80% | ***0.007*** (w) | 5565 | 1624 | -71% | ***0.009*** (w) |
| *Asterias rubens* | 20250 | 7200 | -64% | ***0.010*** (w) | 7686 | 2212 | -71% | ***0.004*** (w) | 44739 | 11012 | -75% | ***0.001*** (w) | 26435 | 5568 | -79% | ***0.000*** (w) |
| *Atelecyclus rotundatus* | 133 | 18 | -86% | 0.075 (t) | | | | | 36 | 0 | -100% | 0.500 (w) | | | | |
| *Buccinum undatum* | 3967 | 359 | -91% | ***0.002*** (w) | 724 | 173 | -76% | ***0.032*** (t) | 1763 | 160 | -91% | ***0.000*** (w) | 6875 | 265 | -96% | ***0.000*** (w) |
| Eggs *Buccinum undatum* | 1062 | 483 | -55% | ***0.000*** (t) | 37 | 25 | -32% | 1.000 (w) | | | | | 831 | 257 | -69% | ***0.007*** (w) |
| *Cancer pagarus* | 1476 | 39 | -97% | 0.188 (w) | 150 | 45 | -70% | 0.500 (w) | 109 | 65 | -40% | 0.548 (t) | 68 | 10 | -85% | 0.156 (t) |
| *Echinoidea* | 77216 | 18159 | -76% | ***0.000*** (t) | 11914 | 1825 | -85% | 0.074 (t) | 60297 | 6704 | -89% | ***0.000*** (w) | 88031 | 6128 | -93% | ***0.000*** (w) |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Hyas araneus* | | | | | | | | | 4016 | 497 | -88% | 0.813 (w) | 112 | 1 | -99% | 0.125 (w) |
| *Liocarcinus depurator* | 1123 | 431 | -62% | ***0.046*** (t) | 846 | 268 | -68% | 0.063 (t) | 1098 | 451 | -59% | ***0.033*** (t) | 419 | 117 | -72% | 0.054 (w) |
| *Liocarcinus holsatus* | 571 | 164 | -71% | ***0.008*** (w) | 1132 | 399 | -65% | ***0.020*** (w) | 1957 | 925 | -53% | ***0.025*** (w) | 2031 | 863 | -58% | ***0.000*** (w) |
| *Liocarcinus marmoreus* | 43 | 15 | -65% | 0.625 (w) | 185 | 54 | -71% | 0.111 (t) | | | | | 144 | 53 | -63% | 0.062 (t) |
| *Macropodia rostrata* | 283 | 72 | -75% | 0.078 (w) | 560 | 67 | -88% | 0.110 (w) | 5044 | 746 | -85% | ***0.005*** (w) | 198 | 58 | -71% | ***0.001*** (t) |
| *Necora puber* | 1109 | 417 | -62% | ***0.014*** (w) | 906 | 463 | -49% | 0.148 (w) | 902 | 262 | -71% | 0.167 (w) | 715 | 154 | -78% | ***0.006*** (w) |
| *Ophiura texturata* | 397 | 21 | -95% | 0.079 (t) | 759 | 119 | -84% | ***0.002*** (t) | | | | | 263 | 52 | -80% | 0.075 (t) |
| *Paguridae* (indet.) | 1298 | 131 | -90% | ***0.002*** (w) | 880 | 105 | -88% | ***0.004*** (w) | 4167 | 309 | -93% | ***0.000*** (w) | 2038 | 50 | -98% | ***0.000*** (w) |
| *Palaemon serratus* | 107 | 61 | -43% | 0.060 (t) | | | | | 1135 | 703 | -38% | 0.168 (t) | 211 | 25 | -88% | 0.125 (w) |
| *Porifera spp.* | | | | | 260 | 236 | -9% | 0.887 (t) | 17405 | 2535 | -85% | ***0.042*** (w) | 220 | 38 | -83% | 0.062 (w) |
| *Sepia officinalis* | | | | | | | | | 2409 | 565 | -77% | 1.000 (w) | | | | |
| **Pooled totals** | | | | | | | | | | | | | | | | |
| Non-commercial fish | | 122 | 44 | -64% | | 1058 | 578 | -45% | | 1238 | 743 | -40% | | 2134 | 424 | -80% |
| Crustacea | | 5050 | 1003 | -80% | | 3802 | 1123 | -70% | | 13483 | 3091 | -77% | | 5512 | 1220 | -78% |
| Echinodermata | | 97864 | 25380 | -74% | | 20360 | 4155 | -80% | | 106410 | 18003 | -83% | | 116273 | 13423 | -88% |
| Mollusca | | 4038 | 381 | -91% | | 848 | 181 | -79% | | 16245 | 3642 | -78% | | 7090 | 281 | -96% |
| All species | | 111205 | 28181 | -75% | | 27544 | 6341 | -77% | | 164295 | 29603 | -82% | | 138638 | 17435 | -87% |

**Table 8.4:** Total numbers of commercial fish in the catch of every panel design for the standard net (A) and experimental net with BRP (B), the percentage difference in catch of the experimental net compared to the standard net (C) and the *p*-value of the Wilcoxon signed-rank (w) or paired sample t-test (t) with significant values in bold italics.

| | 150 mm BRP | | | | 200 mm BRP | | | | 240 mm BRP | | | | 240 mm eBRP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| *S. solea* | 660 | 547 | -17% | ***0.001*** (t) | 438 | 372 | -15% | 0.119 (t) | 1495 | 875 | -41% | ***0.000*** (t) | 909 | 753 | -17% | ***0.003*** (t) |
| *P. platessa* | | | | | 767 | 828 | 8% | 0.125 (t) | 935 | 862 | -8% | ***0.021*** (t) | 92 | 93 | 1% | 0.996 (t) |
| *L. limanda* | | | | | 42 | 45 | 7% | 0.591 (t) | | | | | 205 | 201 | -2% | 0.955 (t) |
| *M. kitt* | | | | | | | | | 134 | 119 | -11% | 0.575 (t) | | | | |
| *M. merlangus* | | | | | 26 | 24 | -8% | 0.910 (t) | 66 | 63 | -5% | 0.806 (t) | 35 | 75 | 114% | 0.875 (w) |
| *T. luscus* | | | | | 71 | 62 | -13% | 0.473 (t) | 360 | 384 | 7% | 0.643 (w) | | | | |
| *G. morhua* | 104 | 84 | -19% | 0.239 (t) | 67 | 60 | -10% | 0.473 (t) | 31 | 44 | 42% | 0.240 (t) | 24 | 27 | 13% | 0.750 (t) |
| *M. surmuletus* | | | | | 179 | 124 | -31% | 0.127 (t) | 616 | 424 | -31% | ***0.000*** (t) | | | | |
| *S. canicula* | 166 | 119 | -28% | ***0.024*** (t) | 157 | 134 | -15% | 0.167 (t) | 857 | 689 | -20% | ***0.010*** (w) | 355 | 302 | -15% | 0.143 (t) |
| *Chelidonichthys spp.* | | | | | 172 | 130 | -24% | 0.403 (t) | 238 | 155 | -35% | 0.075 (t) | | | | |
| *Raya spp.* | 217 | 203 | -6% | 0.686 (t) | | | | | 357 | 344 | -4% | 0.552 (t) | 739 | 723 | -2% | 0.922 (w) |

**Table 8.5:** Total numbers of undersized fish in the catch of each panel design for the standard net (A) and experimental net with BRP (B), the percentage difference in catch of the experimental net compared to the standard net (C) and the *p*-value of the Wilcoxon signed-rank (w) or paired sample t-test (t) with significant values in bold italics.

| | 150 mm BRP | | | | 200 mm BRP | | | | 240 mm BRP | | | | 240 mm eBRP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| *S. solea* | 899 | 622 | -31% | ***0.000*** (t) | 919 | 595 | -35% | ***0.001*** (t) | 2307 | 1194 | -48% | ***0.000*** (w) | 1748 | 880 | -50% | ***0.000*** (t) |
| *P. platessa* | | | | | 2005 | 2097 | 5% | 0.554 (t) | 432 | 367 | -15% | 0.163 (t) | 1359 | 1202 | -12% | 0.087 (w) |
| *L. limanda* | | | | | 127 | 107 | -16% | 0.623 (t) | 42 | 23 | -45% | 0.088 (t) | 2008 | 1721 | -14% | 0.055 (w) |
| *M. kitt* | | | | | 100 | 101 | 1% | 0.958 (t) | 124 | 92 | -26% | ***0.018*** (t) | 36 | 30 | -17% | 0.572 (t) |
| *M. merlangus* | 74 | 76 | 3% | 0.889 (t) | 793 | 557 | -30% | 0.062 (w) | 978 | 633 | -35% | ***0.000*** (t) | 1803 | 1167 | -35% | ***0.002*** (w) |
| *T. luscus* | 90 | 64 | -29% | ***0.028*** (t) | 827 | 643 | -22% | ***0.004*** (t) | 2914 | 2590 | -11% | ***0.041*** (w) | 724 | 289 | -60% | ***0.000*** (w) |
| *Chelidonichthys spp.* | | | | | | | | | 41 | 19 | -54% | ***0.031*** (w) | 107 | 32 | -70% | 0.106 (t) |
| *Raya spp.* | | | | | | | | | 159 | 110 | -31% | ***0.017*** (t) | 146 | 93 | -36% | ***0.008*** (t) |
| Total | 1063 | 762 | -28% | | 4771 | 4100 | -14% | | 6997 | 5028 | -28% | | 7931 | 5414 | -32% | |

The bycatch reduction of undersized sole was highly significant for all BRP's tested not affected by adding the electric stimulus (Table 8.5). It was also much higher than the relative loss of marketable sole. Pouting escaped significantly through all BRP configurations tested, but the escape rate doubled in the eBRP. Employment of a 240mm BRP resulted in a decreased catch of undersized lemon sole and gurnard, although these reductions were not significant for the 240 eBRP. Finally, significantly high bycatch reductions of undersized whiting and small ray were seen in the 240 BRP, both in the presence as in the absence of the electric stimulus.

The length frequency distributions of sole (Figure 2) clearly illustrate the considerable bycatch reduction of undersized sole. It shows that the minimum length for which both trawls tend to catch the same numbers of sole increases with BRP mesh size from 30 cm (150 mm BRP), over 32 cm (200 mm BRP) to 33 cm (240 mm BRP). However, after adding an electric stimulus to the 240 mm BRP, no more differences in catch rates were observed for marketable sole larger of 26 cm and larger. Additionally, the observed loss of marketable sole of 24 & 25 cm shifting from 41% in the 240 mm BRP to 29% in the 240 mm eBRP.

During the eBRP trials, four cod (47, 48, 54 and 55 cm) from the experimental net showed paravertebral hemorrhages during autopsy. Over the entire trial period, in total 52 cod individuals (0.438 ± 0.014 m) were caught in the experimental trawl, resulting in a 7.7% injury rate in cod exposed to the electric cramp stimulus. The dark discoloration of the skin, as well as the location of the spinal injuries was the same as previously reported for this species (Soetaert *et al.*, 2015b; De Haan *et al.*, 2011; Soetaert *et al.*, 2015c).

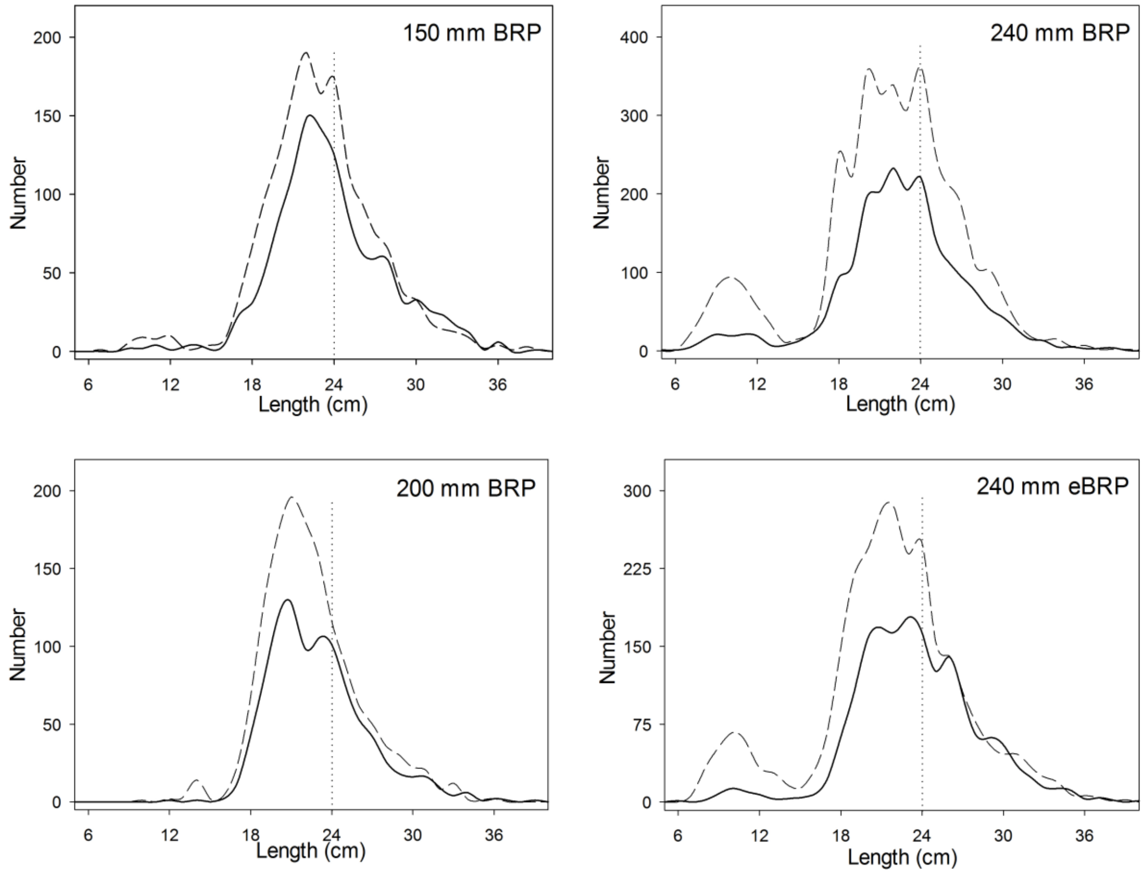


**Figure 8.2:** Length frequency distributions for sole for four different BRP-set ups. Reference net data are given in dashed lines, (e)BRP data in solid lines. The dotted line indicates the minimum landing size (MLS) for sole (24 cm).

# Discussion

## *BRP in traditional round vs square nets*

BRPs tested in the past were rigged into typical chain mat beam trawls (Fonteyne & Polet, 2002; Revill & Jennings, 2005) in which the U-shaped bobbin- and groundrope determine the geometry of the trawl. One of the characteristics peculiar to this trawl design is the presence of considerable slack in the lower panel. Consequently the rigging of a rectangular square mesh panel, that has to be applied fully stretched for optimal performance, is not straightforward in such trawls. Indeed, practice illustrated that slack in front of the BRP caused bag formation and damage to the BRP and the surrounding netting. These issues were not observed during the present experiments, which indicate that no bag formation occurred in the BRPs tested. This result proves that inserting a BRP in a beam trawl with straight bobbin- and groundrope, the so called 'square' trawl instead of a traditional net successfully eliminates these drawbacks. Moreover, only limited abrasion was seen on the chafers, which indicates that the BRPs were positioned well above the seafloor, which facilitates an optimal release mechanism. However, in the 150 mm BRP, limited slack was caused by the fact that the mesh size of the hand knitted 150 mm BRP was larger than intended. Consequently, the panel was slightly longer than the opening in the belly of the net, creating a less stretched geometry of the BRP itself. The extent to which the BRP is stretched inside the trawl undoubtedly affects the release capacity of the panel, as benthos and stones more easily roll over it, while they are otherwise accumulated in front of the BRP or on the BRP increasing its chances to be released. This may be one of the reasons why Fonteyne & Polet (2002) reported an 83% benthos reduction with a 200 mm BRP in a round net, whereas this was only 58% in the present study (Table 8.2). On the other hand, a good match is seen with the benthos release in a 150 mm BRP (70%) and the 67% reduction of the 150 mm BRP with limited slack in the present study. Besides, results of the present study also confirm the finding of Fonteyne & Polet (2002) that body weight was a determining factor, illustrated by significant bycatch reductions of 90% and more of species like whelk and hermit crab.

The observed **loss of marketable sole** in the BRPs tested, was similar to the unacceptably high rates reported by Fonteyne & Polet (2002), but they contrast with the minor sole losses <5% achieved by Revill & Jennings (2005). This discrepancy may be explained by the fact that small marketable sole (24-25 cm), which are the predominant escapees in the present study, are scarce on the fishing grounds where fishing took place by Revill & Jennings (Personal communication, Hans Polet). Fonteyne & Polet (2002) report a commercial loss of sole of 18% with the 150 mm BRP, which is similar to the 17% obtained with the 150 mm BRP, rigged with undesirable slack, in the present study. However, the latter authors observed a 45% loss of sole with the 200 mm BRP, which was much higher than the 15% observed in the present study with a 200 mm fully stretched BRP without slack and equal to the 45% loss found for the fully stretched 240 mm BRP. These results accord to the benthos data, which also showed decreased losses in BRP less slack/bag formation. The same trend, but weaker, was observed for undersized sole, showing a 35 and 45 % reduction for the 150 and 200 mm BRP in a round net respectively (Fonteyne & Polet, 2002), while in the present study this was only 31, 35 and 48% for the 150, 200 and 240 mm BRP respectively. This may probably be explained by the fact that sole attempt to dive as soon as they are caught and stays close to the belly of the net (Fonteyne & Polet, 2002). A stretched BRP demands a more difficult and fast vertical diving movement to escape, whereas bag formation creates escape openings in front of the animal, enabling it to escape horizontally through it. These findings are important, as they indicate that it might be possible to further reduce the 15% loss of sole to an acceptable rate by using a fully stretched 150 or 120 mm BRP, which would allow conventional beam trawls to release large amounts of benthos, debris and undersized sole, with no or minor commercial losses.

The results for plaice, the most important commercial species for beam trawlers by weight (Catchpole *et al.*, 2005) are less consistent. The significant lower catch rates of commercial plaice in the 240 mm BRP contrasts with the (non-significant) higher catch rates in the 200 mm BRP. A similar trend was distinguished for undersized plaice (Table 8.5) and was also previously reported by Fonteyne & Polet (2002). Commercial mullet and large dogfish were caught less in all BRP's, which may indicate that these species tend to swim low and follow the belly of the net, as sole does. The large and significant reductions of undersized fish such as sole, lemon sole, whiting, pouting, gurnards and small rays are important side-effects and may result from the smaller catch volumes enabling a better escapement through the cod-end.

## BRP vs eBRP

Adding an electric stimulus to the BRP (eBRP) did negatively affect the release capacity for **benthos and debris**, as both were lost more in the eBRP. This may have resulted from different benthos composition or the smaller quantities caught in the eBRP trials (Table 8.2), allowing a better release. A more bold explanation might be that it is an indirect effect of the electric stimulus. Flatfish is known to remain low to the bottom (Ryer, 2008) and might therefore block the mesh-openings of the BRP. Applying a cramp stimulus will make the flatfish curl up and be taken away with the water flow, either through the panel, either to the codend, clearing the mesh openings of the panel and reinstate the release capacity. When the effect of electric stimulation on the release of benthic invertebrates is examined, no direct effect can be discerned, with exception for prawn (*Palaemon serratus* L.). This should not surprise as lab experiments determining the behaviour of benthic invertebrates reported either no reaction in Echinodermata, either a retreatment in and subsequent closure of their shell in molluscs, either a cramp reaction resulting in no or hampered movement as observed for crabs (Smaal & Brummelhuis, 2005; van Marlen *et al.*, 2009). Shrimp however, show jump and escape behaviour when exposed to electric stimuli (Polet *et al.*, 2005; Soetaert *et al.*, 2014 & 2015d), which may have promoted escapement through the panel, the side or the back of the net resulting in the observed higher loss.

The electric 80 Hz cramp stimulus had the intended effect on **sole**: no difference in catch rate between the eBRP and reference trawl could discerned for sole larger than 25 cm. The loss of small commercial sole of 24 and 25 cm was reduced with approximately 30%, resulting in a total reduction of lost marketable sole from 41% to 17%. Interestingly, the loss of undersized sole was unaffected. These observations may have three possible explanations. First, small cod escape through the cod-end, which is promoted by the low catch volumes when a BRP is used. This may be valid for undersized sole, but it does not explain the loss of sole of 24 and 25 cm as they should be retained by the 60 mm inner cod-end used. Second, the electric stimulus may not have been strong enough to effectively force all sole smaller than 26 cm in a cramp. However, this is unlikely because the orientation of the animal greatly affects the electric field experienced (Snyder, 2003a) and a relatively sharp cut-off retention size was observed. Nevertheless this might have had an influence, as the strength of the electric stimulus was indeed limited. Although the frequency, pulse type and pulse duration used was the same as used by commercial electrotrawls (Soetaert *et al.*, 2015a), the amplitude was only 47 V instead of 55-60V, the electrodes were also much thinner (12 mm instead of 33) and their intermutual distance was 48 instead of 42 cm. All these parameters contribute to a weaker electric field strength, and thus reaction of the animal. Further experiments are therefore recommended to determine the minimal pulse settings and electrode set-up to affect all sized sole. The third and last explanation is that the smaller sole are indeed also forced in a cramp and bends in a U-form, but are still able to passively fall through the large 12x12 cm meshes when passing the eBRP. Further research is warranted to prove if this hypothesis is correct, by reducing the mesh size of the eBRP from 240 to 200 mm and examining if the cut-off retention size shifts to 24 cm, the minimum landings size of sole. Besides, also video recordings of the panel during trawling seem extremely valuable to include in further field trials in order to get a better insight in the release mechanisms and fish behaviour in relation to (e)BRPs.

The electric pulse stimulation seems to have no effect on the catch of most **undersized fish** species. Species like plaice, whiting, and raya spp., of which sufficiently large numbers were caught, show similar reductions in the 240 mm BRP and the 240 mm eBRP. This may indicate that their loss was attributed to increased escapement through the cod-end, promoted by the smaller catch volumes, which were similar for both net designs. However, the results for these species contrast with those of pouting, demonstrating an increase in reduction from 11 to 60%. This may point to a reinforced escape response as a consequence of the electric stimulus. Since it is unlikely that small pouting is not immobilized by the pulse and passes through the panel, the observed loss might result from escapement through the side and back of the net after being startled by the electric pulse. Similarly, physical and visual stimuli have also been reported to promote fish escape behaviour and increase the selectivity of panels in trawls (Glass & Wardlle, 1995; Kim & Whang, 2010; Herrmann *et al.*, 2015). This increased escape behaviour was not observed in the whiting. This might be caused by whiting's upward flight behaviour (Ferro *et al.*, 2007; Krag *et al.*, 2009) as a result of which it does not encounter the electric field. These findings emphasize the importance of video recordings in future research in order to fully unravel and understand underlying mechanisms.

Finally, the implementation of the electric pulse had **unwanted side-effects** on cod, showing paravertebral hemorrhages in 7.7% of the 52 animals. This incidence was lower than the previously reported 9-11% (van Marlen *et al.*, 2014) which may result from the reduced electric field strength (de Haan *et al.*, 2009) or the reported strongly variable sensitivity of this species (Soetaert *et al.*, 2015c).

## *Reduced environmental impact*

Bycatch and discard mortality accounts for only 5-10% of the total benthic mortality caused by beam trawling (Lindeboom and de Groot, 1998), as most animals die as a consequence of direct mortality or damage in the trawl path of commercial flatfish trawls (Bergman & Santbrink, 2000; Kaiser *et al.*, 2006), which is not affected by the presence of a BRP inside the trawl. However, several benthos species will be positively affected as they drop out directly from the trawl, at the site of capture without additional stress and harm suffered in the cod-end or on board processing. According to Revill & Jennings (2005) survival rates for many BRP escapee crustaceans, molluscs and echinoderms are close to 100% and they conclude that the implementation of a BRP can reduce the overall environmental impact expressed as invertebrate mortality of beam trawl fisheries by 5-10%.

In contrast to a relatively high discard survival of invertebrates in the beam trawl fishery, the survival rates of discarded undersized fish of commercial species are generally low: 0 - 50% for Pleuronectiformes, over 70% for Rajiformes and 0% for whiting and pouting (Van Beek *et al.*, 1990; Benoit *et al.*, 2012; Depestele *et al.*, 2014), stressing the importance of minimizing the bycatch of undersized fish. The numbers given in Table 8.5 are therefore promising, and probably an underestimation because the in-liners used in the present study hamper the escape of undersized fish. Moreover, the survival chances of the bycaught undersized fish will also grow as a consequence of smaller catch volumes (Depestele *et al.*, 2014) and subsequent shorter on-board processing time, resulting in less stress and harm (Davis, 2002).

### ***Promising perspectives***

Looking from a fisherman's perspective at the distinctive advantages of BRPs, the bycatch reduction of undersized fish may become far more important than the drop-out of unwanted benthos. From 2016 on, discarding will be prohibited in Europe and fishermen will be obligated to land undersized fish of commercial species, directly influencing TAC and quota allocation (NSAC, 2014). The implementation of (e)BRPs may therefore become increasingly beneficial, as it can possibly help to reduce unwanted bycatches. In addition, smaller catch volumes as a result of a lower benthos and debris proportions may result on one hand in a better bycatch survival and on the other hand in better fish quality, due to diminished damage in the codend and consequently a better market price. These both economic and environmental benefits offer promising opportunities and an incentive for further optimization and investigation of BRPs.

In conventional beam trawls, a BRP without added electric stimulation is the most obvious to implement as it does not require any investment in electric devices. Compared to an eBRP, the mesh size would have to be smaller to compensate the commercial loss of sole, leading to lower bycatch reductions of benthos, debris and undersized fish. Further BRP research should focus on determining the ideal mesh size allowing sufficient bycatch drop-out and the retention of adequate commercial catches of sole.

On the other hand, when dealing with electrotrawls the step to implement an electrified BRP is relatively small since the electric equipment is already installed in the fishing gear. This would enable the use of larger eBRP meshes, resulting in higher loss of benthos debris and undersized fish, without extra loss of marketable sole. In practice the relative bycatch reduction of benthos and debris will be smaller than observed in this study, as the amounts of benthos and debris entering the net of an electrotrawl is much smaller due to the elimination of tickler chains or chain matrices. However, a better retention of commercial sole is required first to enable a successful commercial introduction. Therefore, further research should focus on an optimized electric stimulus and adjusted BRP mesh size. First, the minimal cramp stimulus that prevents sole from diving should be determined. Previous studies (Stewart, 1977; Soetaert *et al.*, 2015b) report that lower frequencies still result in cramp reactions, which would permit smaller distance between the electrodes for the same power, resulting in a more homogenous distribution of the electric field. Besides, lower frequencies may also decrease cod injuries (De Haan *et al.*, 2011). If this research points out that the pulse settings or electrode set-up was inadequate to retain all commercial sole, additional field trials with a 240 mm eBRP and the optimal configuration should be performed. Otherwise, the mesh size of the eBRP should be decreased to 200 mm. The implementation of a cramp stimulus should be sufficient to completely eliminate the 15% loss of commercial sole observed in the 200 mm BRP, if a similar increase in retention is achieved as observed for the 240 mm (e)BRP. Finally, future field trials should include underwater video recording of the fish behaviour in relation the (e)BRP. Even though this requires clear water conditions which may affect the fish behaviour, it is the best and fastest way of obtaining evidence and learn if fish is actively diving through the panel or passively going through, if flatfish are blocking the meshes of the BRP, if an electric stimulus is indeed promoting escape behaviour in undersized pouting, if there is a size or species effect of the electric stimulus, …

# Conclusion

Implementing a BRP in a 4 m beam trawl with rectangular chain mat, straight bobbin- and groundrope eliminates the issue of suboptimal rigging resulting in slack and subsequent damage. The release of benthos, debris and fish tends to increase with mesh size, but appears to be lower in a stretched panel compared to one with slack. A significant decrease in the bycatch of undersized fish of commercial species was observed in all (e)BRPs tested, irrefutably demonstrating its capability to reduce both invertebrate and to allow undersized fish to escape underwater. However, all BRPs still showed unacceptable loss of commercially important sole. Adding an electric stimulus to the panel drastically decreased the sole loss, by provoking a cramp reaction in sole passing over the panel, without negatively affecting the release of benthos and debris. Further research should therefore focus on a complete retention of commercial sole by optimizing the electric stimulus and/or reducing the panel's mesh size.

# CHAPTER 9

# GENERAL DISCUSSION

## Overview of the results

Part I: Assessing the safe range of electric pulses for **invertebrates**.

- Sandworm did not show increased mortality or injuries 14 days after exposure in a homogenous electric field to pulses with high frequencies (200 Hz), electric field strengths (200 V $m^{-1}$), pulse duration (1000 μs) or exposure time (5 s, 4x1 s) tested. There was also no effect of pulse type (PAC, PBC) or pulse shape (exponential, quarter sinus) found.
- Brown shrimp did not show increased mortality or injuries 14 days after exposure in a homogenous electric field to pulses with high frequencies (200 Hz), electric field strengths (200 V $m^{-1}$), pulse duration (1000 μs) or exposure time (5 s, 4x1 s). However, there was a significant increase in viral bodies in the haepatopancreas of shrimp exposed to the highest field strength (200 V $m^{-1}$).
- Repetitive exposure (20 times in 4 weeks) did not reveal differences in survival, moulting behaviour or egg release between shrimp exposed to the commercial electrodes generating electric stimuli (cramp pulse for sole and startle pulse for shrimp) or shrimp exposed to mechanic (tickler chain) stimulation after 14 days. However, shrimp exposed to the sole pulse showed significant lower survival compared to one of the two non-exposed control groups whereas shrimp exposed to the mechanical stimulus showed significant less moulting and a higher mortality for large shrimp in comparison to the same non-exposed control group.

Part II: Determining the safe range of pulse settings for **flatfish**.

- Sole did not show increased mortality or injuries 14 days after exposure in a homogenous electric field to 47 different electric pulses in different orientation. All pulse parameters were varied to the same maxima as used in the experiments with sandworm and tested at different frequencies. There was also no effect of pulse type (PAC, PBC), pulse shape (exponential, quarter sinus, sinusoidal) or orientation. Therefore, it is very unlikely that varying and increasing pulse parameters in commercial fishing practice, within the range tested in the present study, would result in injuries or mortality in sole.

Part III: Investigating (the role of pulse parameters in) the sensitivity of **roundfish**

- Exposing cod between plate shaped electrodes has a larger impact on the animal. This was illustrated by the much lower thresholds for the occurrence of epileptiform seizures.
- No conclusive information on the effect of different parameter settings could be demonstrated due to a lack of injuries observed.
- Under identical experimental settings, the sensitivity of cod for developing spinal injuries as a result of exposure to electric pulses varied between 0 and 70%. Although it was suggested that rearing conditions might play an important role, no decisive morphological or physiological parameter explaining the observed variability could be evidenced.
- Seabass exposed under the same experimental conditions as cod did not show injuries or mortality 14 days after exposure. This may indicate that electric-induced spinal injuries as observed in cod and whiting are a typical gadoid/cod species effect rather than a general roundfish-effect. It was hypothesized that differences in the number and size of vertebrae may affect the mechanical strength and susceptibility for spinal injuries.

Part IV: electric pulses' potential for **further innovation** and increased selectivity

- The square electrotrawl design seems to be well-suited for the implementation and reliable use of benthos release panels.
- By adding electric stimuli to the benthos release panel in front of the cod end, the loss of marketable sole could be strongly reduced, without affecting the loss of benthos and debris. Moreover, significant lower amounts of undersized fish were caught. The present results approve that electric pulses enable new promising opportunities for a better catch selectivity.

## Implications of the experimental set-up

When the impact of electric pulses on animals is investigated, the laboratory set-up has a major effect on the generated electric field in different ways. Firstly, an electric field generated in experimental tanks will differ from the field generated at sea because the walls of the experimental tank prevent electric charge to disperse. As a consequence, an equal potential between electrodes requires less electric power and current in a laboratory set-up. This results in reduced electric current flow or increased field strengths for a potential and current driven generator, respectively. Secondly, the lack of a deep conducting substrate (like the sea bed) in a tank is reinforcing the electric field above the floor. Thirdly, numeric modeling demonstrated that the presence of other animals in the same electric field is increasing the electric field experienced by a single individual (D'Agaro & Stravisi, 2009). Therefore, in our experiments with invertebrates, a high number of individuals were present in the same tank, to obtain a worst-case scenario. Fourth, the choice between plate electrodes (inducing a homogenous electric field) and wire-shaped electrodes (inducing a heterogenous electric field) results in completely different electric fields, and will therefore always be arguably. The use of plate electrodes has the advantage that variation in the experienced electric field is much easier to control, which is important when investigating different pulse parameters. The wire-shaped electrodes on the other hand, accord much more to the field situation, but require a standardized position of the animal. However, both types of electrodes result in different electric field distribution around the animal. This may also affect the physiological impact, which was illustrated in our studies (Chapter 5-6) by the lower threshold for epileptiform seizures in a homogenous set-up. However, it remains to be elucidated how the effect of a certain constant amount of electric current passing through the whole body of an animal (homogenous field) differs from an exponential decay over the body ranging from very high to low amounts of electric current (heterogenous field).

Further research assessing the relation between electric pulses and the reaction of fish should include numerical simulations of the electric current flowing inside the fish body (A $cm^{-2}$), as this may be more explanatory than the field strength expressed as V $m^{-1}$ in the water column. Indeed, although a flatfish and round fish may experience the same potential difference, more electric charge will flow through a larger more voluminous round fish body. This is most likely the reason for the good inverse correlation between fish volume and the threshold for epileptic seizures observed in Chapter 5 and experiments reported by Dolan & Miranda (2003). A model that correctly estimates the electric flow in and through the fish body may be very successful in predicting the related fish behaviour and reactions. Modeling the fish's body as an entirety consisting of different tissues with particular electric characteristics would provide insight in the distribution of electric current in the fish's body, and which parts of the body receive the highest electric load. Ideally, this process should involve lab experiments determining the electric resistance and inductance of each tissue in function of frequency and amplitude. Subsequently, the boundaries of the model can be enlarged from an isolated fish in a small volume of water to one or many fish exposed at different spots in a heterogenous set-up. This will require many experiments for calibration data, which will have to include extensive measurements of both pulse settings and environmental variables such as temperature and conductivity. The effect of pulse parameters is illustrated by neurologic research, showing that e.g. the threshold of epileptic seizures is a function of voltage, pulse type, pulse shape and frequency (Weaver *et al.*, 1974; Weiner, 1988; Kelner, 1996) as discussed in Chapter 5. Temperature is often reported to have a major impact on the fish's (behavioural) response, being positively correlated with activity, reaction speed, endurance, … (Winger *et al.*, 1999; Ozbilgin & Wardle, 2002; Ryer, 2008). Water conductivity is illustrated by Lines & Kestin (2004) to strongly affect the electric flow inside the fish body: whereas in seawater the electric field inside the fish is approximately 50% of that in the surrounding water, it is 200% in fresh water. Finally, linking the current distribution in the fish's body to specific reactions may also give us indications of how the stimulation of certain parts of the body/brain/nerval system/muscle relates to physiological reactions. This can be supported by lab-experiments in which different slices of the fish's body are electrically isolated from another in the water column. Exposing only one part while shielding of the rest of the fish's body from electric current entering from the outside/water would allow us to unambiguously correlate the electrical impact on a certain part of the body or brain to the physiological reaction (in another). Improving this fundamental knowledge is crucial to fathom the observed effects and to unravel the interaction between epileptiform seizures with/without spinal injuries and vice versa, which may be triggered by different (independent?) pathways.

## Assessing and controlling the direct impact of electric stimulation in the field

Except catch comparisons, research directly assessing the impact of electrotrawls is limited to the study of Depestele *et al.* (2015), reporting smaller alterations of the seabed bathymetry compared to conventional beam trawls, which is likely to be a result of a lower penetration depth. Besides, a sudden increase in the number of dab demonstrating skin ulcerations was found at the same moment electrotrawls were introduced, which was considered as a causal relationship (and interpreted as skin burns) by some fishermen (Devriese *et al.*, 2015). However, the incidence of ulcerations did not increase with increasing numbers of electrotrawls, and the first results of 2015 indicate that the incidence may be decreasing, despite unchanged fishing efforts by electrotrawls (personal communication, Lisa Devriese, ILVO Ostend, Belgium). Furthermore, De Haan and coworkers (2015) were not able to induce skin ulcerations in dab after direct electric stimulation. Also in our studies, skin ulcerations were not observed in electrically exposed fish. This points towards a lack of proof that direct skin lesions are induced by electric exposure. However, no data are available concerning the presence of micro-lesions or other indirect effect (e.g. secondary bacterial infections) in the exposed animals, requiring further investigation and follow-up of this phenomenon.

Previous exploratory studies with large numbers of *invertebrate species* concluded that the impact of electrotrawls on invertebrates is expected to be smaller compared to conventional beam trawls (Smaal & Brummelhuis, 2005; van Marlen *et al.*, 2009). The presented elaborate studies with brown shrimp and ragworm in an experimental set-up (Chapter 3) and brown shrimp in a veracious set-up with commercial wire-shaped electrodes (Chapter 4) could not refute this statement, but indicate that caution is warranted. Indeed, the first homogenous study with a homogenous showed an increase in IBV infection in shrimp exposed to field strengths of 200 V $m^{-1}$. Although this was not confirmed in the study with the heterogenous approach, the latter revealed a decreased survival of shrimp exposed repetitively to the cramp pulse for sole compared to one of two tested non-electric-exposed control treatments. However, no difference in survival was found with mechanically stimulated or non-stressed shrimp. It can be speculated that the reduced survival of shrimp exposed to the sole pulse in the laboratory has no major negative implications for commercial fishing practice. First because the shrimp in this study were exposed 20 times in 4 days, whereas only 0.6% of the seabed is estimated to be trawled more than 20 times a year (Rijnsdorp *et al.*, 1998) and less than 20% survives longer than 1 year (Oh *et al.*, 1999). This indicates that it is very unlikely that shrimp would be exposed to the sole pulse so frequently in the field, and definitely not in such a short time interval. Second, this difference was not observed in the homogenous experiment in which shrimp were exposed up to four times to the sole cramp pulse. Although the number of exposures was lower, all shrimp in the homogenous study were exposed with their entire body to high field strengths for the entire exposure time, which was not the case in the heterogenous set-up. Third, no difference in survival was observed with the non-stressed shrimp. This suggests that even if there is an effect a possible effect of this worst case scenario will be limited because a major effect would have resulted in significant differences with the other treatments as well. Fourth, the significant lower amount of moults and reduced survival of large animals exposed to mechanical stimulation did not result in a provable increase in mortality after 14 days, but they may contribute to a decreased recruitment in a longer term. Finally, electric stimulation would replace mechanical stimulation, which is therefore a more appropriate reference treatment than non-stressed animals when assessing the net impact in commercial fishing practice. Indeed, the crucial question is if a shift from mechanic to electric stimulation in beam trawl fisheries will negatively impact shrimp (stocks), but so far none of the experiments did provide evidence of such trends.

Exposing non-commercial *fish species*, as well as sole, plaice and cod, to the startle pulse for shrimp only had limited immediate impact on the animals (Desender *et al.*, 2015), being small focal haemorrhages in plaice and an increase in melanomacrophage centers in a few cod 1 day after exposure. However, these differences were not observed in cod and sole, 14 days after being exposed to much more intense pulses (Chapter 5), indicating that if such minor lesions would occur, animals recover relatively fast. Additionally, the short quivering reactions observed in sole immediately after exposure (Chapter 5) are not likely to occur in electrotrawls. Indeed, first of all, such events only occurred at pulse settings far beyond commercial practice. Secondly, the threshold to induce such seizures is even much higher when commercial wire-shaped electrodes are used (Chapter 5-6). It may therefore be argued that the electric stimulation used by pulse trawlers will not have severe direct negative effect on sole. The same statement can be made for electro-sensitive animals, which's exposures to commercial electrotrawl pulses did not result in macroscopic injuries, mortality or reduced food response (De Haan *et al.*, 2009a).

These results contrast with the studies involving *cod*. Indeed, paravertebral haemorrhages were observed in 7-11% of cod caught in the field by electrotrawling (van Marlen *et al.*, 2014; Chapter 8) and in 0-70% of the animals experimentally exposed near the electrodes as demonstrated in our studies (Chapter 5 & 6) and by others (De Haan *et al.*, 2011). Investigating the key-factors for a reduced impact of electrotrawls on cod require a set-up that mimics the field as closely as possible, as done in the heterogenous experiments with cod, sea bass and shrimp (chapters 4, 6 & 7). However, it should be realized that it remains difficult to correctly estimate the net impact of the interaction between fish, electricity and set-up related parameters as mentioned above, as for example electrotrawl gears are not stationary but towed and different designs of electrodes may occur. This should be taken into account when interpreting the heterogenous experiments with cod and sea bass (Chapter 6 & 7). An underestimation of the experienced electric field may result from the fact that the isolated part of the electrodes in the laboratory measured 0.6 m, while those in the field are alternating 0.6 and 0.25 m long. This implies that if an adult cod (MLS>0.35 m) is passing a short isolator in the field, it may be stimulated by two pairs of conductors simultaneously, which may increase the impact. On the other hand, some factors may have promoted a stronger electric stimulation compared to the field. Firstly, the distance between two electrodes in our experimental set-up was always 0.32 m, while commercial vessels targeting sole have a distance of 0.42 m nowadays. This reduction in inter-mutual distance results in higher field strengths in the laboratory set-up, although this will be partially counteracted by the increase in electrode diameter from 26 mm (experiments) to 32 mm in the field. Second, the rubber discs limiting the conductors were removed during the tests with F2, F3 and W cod (Chapter 6). Consequently, these animals were positioned in close association to the conductors in the zone of maximal field strength, which will not be achieved in a commercial electrotrawls. It can therefore be concluded that the field strengths to which cod was exposed in the experiments of de Haan *et al.* (2009b, 2011) and Chapter 6 and 7 are representative for the electric field near the electrodes of electrotrawls. However, animals are randomly allocated to the electrodes in commercial fishing practice. As a result, most fish will not be subjected to such large fields and show less/no injuries. Indeed, de Haan *et al.* (2011) demonstrated that animals located over 0.2 m above or over 0.4 m sideways of the electrode pair was never injured. This probably explains why an injury rate of ± 10% was found in the field (van Marlen *et al.*, 2014), because only a minority of the animals is effectively exposed in the zone of maximal field strength in the field. The upward flight behaviour of whiting (Ferro *et al.*, 2007; Krag *et al.*, 2009), reducing the chance of being exposed near the electrodes, may be (part) of the reason why this species showed only ± 2% injuries in the field (van Marlen *et al.*, 2014). It is therefore important to always nuance that the 0-70% of cod showing spinal injuries in lab exposure studies near the electrodes do not reflect the injury rates provoked in the field.

For many years, avoiding *spinal injuries in Gadoids* has been a major challenge of pulse fishing. As they result from powerful contractions during a cramp reaction, it can be argued that using a different non-cramp electric stimulus would eliminate this drawback. However, it is this cramp reaction that renders this technique very efficient to catch sole, so changing the pulse settings to avoid adverse effects in cod will most probably result in reduced catch efficiency for sole. A exiting and promising solution was suggested by de Haan *et al.* (2011), who demonstrated that the number of injuries was decreased to zero if the frequency was raised to 180 Hz (duty cycle of 2.9%). Similarly, Roth *et al.* (2004) reported a maximum number of injuries when stunning (100% duty cycle) Atlantic salmon at a frequency of 50-80 Hz and a decline with higher frequencies up to 500 and 1000 Hz. As the results presented in Chapter 5 prove that sole also exhibit a strong cramp reaction at frequencies up to 200 Hz, these findings are a call for further investigating the effect of pulses in the 150-200 Hz frequency range on cod. If the above results are confirmed, the use of such cramp pulses on electrotrawls may considerable reduces the damage inflicted to cod and other gadoid species.

Although spinal injuries may be reduced by adjusting the electric pulse parameters (Sharber, 1994; Dalbey *et al.*, 1996; Snyder, 2003; Schreer, 2004; De Haan *et al.*, 2011), the results presented in Chapter 6 demonstrate that the pulse settings are subordinate to the unknown fish-specific parameters that define the sensitivity of the fish. As a consequence, it is unlikely that one will succeed in fully eliminating spinal injuries in Gadoids, as long as cramp stimuli are used in electrotrawls. Therefore, other techniques should be designed. One possibility might be to fence gadoid roundfish off from entering the intense electric fields. However, this will be challenging, because cod has the preference to enter trawls close to the seabed (Ferro *et al.*, 2007; Krag *et al.*, 2009; Herrmann *et al.*, 2015) and is reported to be disoriented in electric fields (Stewart, 1973). This reflex is not seen in other gadoids like haddock and whiting which have a vertical preference of swimming (Ferro *et al.*, 2007; Krag *et al.*, 2009) away from the electrodes. The latter may also explain the lower incidence of spinal injuries observed in whiting. Therefore, a technique could be designed to abort cod's diving reflexes and induce an upward movement. This may be done by the implementation of lighting to the electrodes or phosphorescent large meshed netting material to disrupt their behaviour. Another possibility is fixing an upward panel that starts just above the seafloor at the beam and goes to the end of the electrodes allowing benthic fish to go underneath but forcing round fish up. Finally, 'cod repelling devices' that expel cod from the beam opening may be successful too. The use of light on the beam, phosphorescent large meshed netting material in the opening under the beam or sweeps pointing to the front herding round fish away from net opening, are therefore interesting topics for further research.

Although every effort should be made to avoid injured Gadoid fish, this drawback should be traded off with the beneficial effects of replacing the mechanical by electric stimulation. Indeed, electrotrawls catch substantially less discards (Rasenberg *et al.*, 2013; van Marlen *et al.*, 2014) and have a reduced bottom impact (Depestele *et al.*, 2015) which is expected to result in a reduced trawl path mortality (Teal *et al.*, 2014), which imply an improvement in animal welfare for unaffected cod and all other species. Moreover, they show a drastically reduced fuel consumption (Poos *et al.*, 2013). Subsequently, electrically induced injuries in commercial cod may be considered as mainly an ethical issue, since injured or not, they are caught and slaughtered and the economic loss is largely compensated by the fuel reduction. A very important question here is whether injuries are also inflicted to juvenile and undersized cod. Such animals are discarded or can escape the net through the cod-end and therefore may suffer intensively and demonstrate reduced growth rates, condition and survival (Dalbey *et al.*, 1996). Note however, the impact on undersized cod should also be put in perspective for two main reasons: (i) cod is less often caught by electrotrawls compared to conventional beam trawls (van Marlen *et al.*, 2014) and (ii) the catch weight and towing speed of pulse trawlers is lower compared to conventional beam trawls, which reduces the mechanical impact experienced by the animals, resulting in a better survival (Depestele *et al.*, 2014). Therefore, the impact on juvenile animals is largely depending on the extent of the induced injury. In this respect, studies dealing with juvenile cod are crucial. However, the few studies that have been performed show contradictory results. De Haan *et al.* (2011) did not observe injuries in small juvenile cod (0.12-0.16 m) that were exposed repetitively, while van Marlen *et al.* (2011) found paravertebral haemorrhages (indicating spinal injury) in 3 out of 5 wild cod smaller than 28 cm, or an average 22% for all undersized wild cod caught. These differences could be attributed to differences in fish-related parameters such as muscularity, bone mineralization or breeding effects. Indeed, the nonharmed small juveniles used by de Haan *et al.* (2011) were intensively reared in the same way as the insensitive F1 and F2 cod that showed no or low injury rates (Chapter 6). For this reason, the statement of van Marlen *et al.* (2014) that “electric pulse fishing will not affect juvenile cod stocks” should be reconsidered. Indeed, examining the effect of commercial electrotrawls on juvenile and undersized cod may be the most critical and urgent command, since an electrotrawl fleet as currently operating in the Netherlands might be a threat for the cod population if these animals would be systematically harmed. Therefore, extensive examination of the catches of electrotrawls targeting sole, using an inner cod-end to retain small juvenile cod and whiting, is warranted. Moreover, laboratory exposure studies with different size classes of (extensively reared) undersized cod are recommended.

# Total impact of electrotrawls

When discussing the impact of beam trawling, both conventional and electrotrawling, one should be aware the gears targeting flatfish or shrimp show important differences. Firstly, beam trawls targeting flatfish are equipped with chain(matrice)s ploughing the seabed whereas those targeting shrimp have bobbins rolling over it, which results in less intensive bottom contact and lower fuel consumptions for the latter trawl. Secondly, all chains except the footrope are forbidden when electrodes are used in a flatfish gear, whereas no restrictions are imposed for the shrimp gear. As a result, both trawls affect their environment differently and ask for particular management. Therefore, the impact of a possible transition from a conventional beam trawl to an electrotrawl is discussed separately for sole and shrimp.

## *Electrotrawl targeting sole*

The *impact on invertebrates* is caused mainly by the induced trawl path mortality and to a minor extent by damage encountered when bycaught (Bergman & Santbrink, 2000). The largest impact occurs in the trawl path, either as a direct result of physical damage inflicted by the passage of the trawl or indirectly owing to disturbance exposure and subsequent predation (Bergman & Santbrink, 2000). The trawl path mortality was found to be 5 to 40% of the initial density for gastropods, starfishes, small and medium crustaceans and annelids and up to 80% for bivalves (Bergman & Santbrink, 2000). The trawl path mortality induced by electrotrawls targeting sole may be smaller for three reasons. First of all, electrotrawls are towed at 4.5-5 kn, which is slower than the 6-7 kn of conventional beam trawls with tickler chains (Rijnsdorp *et al.*, 2008). This 10-25% decrease in towing speed implies a proportional decrease in trawl path length per unit of time and thus benthos encountered. Additionally, the lower catch volumes and reduced amount of debris will likely reduce the damage inflicted to caught benthos in the cod end. Secondly, electrotrawls are not allowed to have chains other than their footrope in front of the net. Models showed that this results in a reduced penetration depth (Teal *et al.*, 2014), which explains the differences in bathymetry reported by Depestele *et al.* (2015). This decreased number of chain passages and bottom contact intensity is also likely to lessen the impact on benthos. It is therefore speculated that the replacement of the chains by electrodes also reduces trawl path mortality, but this was not yet demonstrated (Teal *et al.*, 2014). Thirdly, as the recovery rate and time of benthic communities are closely aligned with physical disturbance (Dernie *et al.*, 2003a&b), benthic communities may recover better and faster from the passage of an electrotrawl, which has a lower physical impact than conventional beam trawls. However, electrotrawl may also have a larger impact in some situations as they can fish soft muddy substrates that were not impacted by conventional beam trawls in the past. In contrast to the restoration times of sandy habitats that are dominated by physical processes (days to a few months), muddy habitats are mediated by a combination of physical, chemical and biological processes resulting in much slower habitat restoration (months to >1 year) (Dernie *et al.*, 2003a&b). As a consequence, the associate biota is much more vulnerable, with predicted recovery times measured in years (Kaiser *et al.*, 2006). The impact of electrotrawls fishing on these soft sediments has not yet been determined but may be larger than that of a conventional beam trawl fishing on sandy substrates. Finally, benthos species are also affected when by-caught. Short-term catch comparisons of electrotrawls targeting sole described by van Marlen *et al.* (2014) revealed 61.6 % fewer benthos discards in weight per hour trawling compared to mechanical stimulation. A more elaborate catch monitoring program by Rasenberg *et al.* (2013) had a more conservative finding, revealing a 16 % and 42 % decrease in numbers of starfish and crabs when electrotrawling, respectively. This reduction in discards may have a positive effect, although the impact will be more limited as benthic invertebrates generally survive the discarding procedure (Depestele *et al.*, 2014). In order to obtain a correct estimate of the net-impact on invertebrates, impact studies should assess the effect of electrotrawls on the recovery of different habitats and the associated organisms. These data can then be combined with the existing impact studies on beam trawls to calculate and compare the net impact of both gears based on the fishing effort of each fleet in certain habitats.

The *impact of electrotrawls on fish* is contradictory but replacing electric by mechanical stimulation seems to result in a decreased impact on undersized commercial fish. Van Marlen *et al.* (2014) found a significant 21% decrease in marketable sole and a 43.9% reduction in fish discards measured in weight per hour in a 33 hauls comparison experiment. In contrast, Rasenberg *et al.* (2013) reported a 10-20% increase of marketable sole, but only minor non-significant lowering effects on plaice and sole discards during a sampling program including 50% of the electrotrawl fleet. These differences may be explained by spatial variation as observed by Rasenberg *et al.* (2013) and by the fact that the electrotrawls in the study of van Marlen *et al.* (2014) were not fishing on their familiar and well known fishing grounds, which may have resulted in smaller catches. Nevertheless, both studies demonstrate a reduction when the discard rates are expressed as kg fish discards per kg marketable fish. In addition, electrotrawls have smaller catch volumes and invertebrate bycatches, which may improve the survival chances of the caught undersized fish (Depestele *et al.*, 2014) and the subsequent shorter on-board processing time will result in less stress and damage (Davis, 2002).

Finally, a fleet of electrotrawls may have *(in)direct biological effects on the foodweb*. On the one hand, a reduced mortality of undersized fish may positively affect the fish stocks, and on a longer term the quota and earnings of the fisherman (Revill *et al.*, 1999). On the other hand, reduced trawl path mortality and fish discard mortality negatively affect the food supply for scavengers on which fish predate, thus reducing their food supply. Modelling these effects and possible interactions are required to correctly estimate the net effect. Therefore, long term observations and more elaborate data sets, especially from catch volumes, catch composition and the (new) fishing grounds used, are warranted for a correct calibration and a reliable interpretation. This does not necessarilly requires time, effort and money consuming monitoring programmes, since a lot of data is already being collected. Landing and vessel monitoring system (VMS) data are available for all commercial vessels. The data of electrotrawls can be analysed and compared on one hand to historic data of the same vessel when it used mechanical stimulation and on the other hand with conventional beam trawls fishing on the same spots. Such research can be carried out quickly and relatively cheap, but would nevertheless disclose valuable information on (species specific) differences in catch efficiency, changes in fishing effort, shifts to new fishing grounds, …

## *Electrotrawl targeting shrimp*

Despite the impact of the shrimp startle pulse has been examined on invertebrates (Chapter 3, 4), different species of flatfish, non-commercial fish and roundfish (Chapter 5, Desender *et al.*, 2015), no alarming side-effects have been reported. This suggests that gear-effects will be decisive in the assessment of the total impact. As a result of the straight bobbin rope with reduced number of bobbins and the potential to raise the footrope in electrotrawls targeting shrimp, the total volume of fish and benthos by-catch may be reduced with 50 to 76% (Verschueren *et al.,* 2014). An indirect long term effect of this bycatch reduction of commercial fish, is a potential increase of the fish stocks and total allowable catches (TAC), resulting in higher landings and earnings (Revill *et al.*, 1999). For example, it may be estimated that only the bycatch reduction of small plaice of minimum 30% (Verschueren *et al.*, 2014), will accord with a 3-7% increase of the TAC for plaice in the North Sea (Revill *et al.*, 1999) or lost landing valuing about €6 million a year. However, as shrimp is escaping the same way as the by-catch does, minimal discards have to be traded off with maximal shrimp catches. Therefore, fishermen and management may aim for two scenarios. On the one hand, the number of bobbins may be minimized with a maximal elevated footrope, resulting in minimal by-catch but similar shrimp catches as conventional beam trawls. In this scenario, fishermen have no economic advantage to justify the large investments, except when they would be allowed to fish in protected areas that conventional beam trawls can't access. On the other hand, a maximal number of bobbins and low footrope with minimal escape opportunities may be used, resulting in equal by-catches but much higher shrimp catches compared to conventional beam trawls. In both scenarios however, a huge reduction in the ratio by catch/marketable shrimp is realised. Nevertheless, a better insight in the catch composition and fishing effort of these vessels is desirable, especially for those electrotrawls that maximise the number of bobbins and their total catch which have not been monitored yet.

## *Regulation and enforcement*

A good impartial legislation and control defining the maximal allowed pulse outputs and the gear design is crucial for several reasons. First and most important, it prevents that manufacturers and fishermen can abuse this technique, either combining both mechanical and electric stimulation, either by increasing the strength of the electric pulse. This can result in (more) unwanted side-effects in organisms but also in a too large fishing effort that threatens the stock as observed for shrimp in China (Yu *et al.*, 2007). Second, the comparison and interpretation of catch data, landings or trends in stocks over a couple of years are less useful and instructive if the gear and pulse settings are unknown or increase over time. Last, because most effect studies done so far were limited to the pulse (gear) settings currently used. Allowing the industry to use stronger pulses implies that the presented results are no longer representative. The latter issue can be tackled by (proactively) doing further experiments to determine the safe range of pulse settings as done in this thesis and/or model the possible side-effects in function of the pulse settings used. The current European legislation imposes an upper limit of 15 Vrms, which can be interpreted as an 'average voltage'. However, this is inadequate as it still enables the use of pulses with amplitudes over 500 V and allows electrotrawls another 50% increase compared to their current setting, which are both undesirable. It would therefore be more appropriate to provide a maximum duty cycle, peak voltage and power output per meter beam based on the exposure experiment performed, with e.g. a 20% margin. Besides, a minimal distance between the electrodes and the prohibition on mechanical stimulation different than the footrope of the net should be included. These limitations would prevent misuse but still provide enough space for further optimization and the development of new applications.

## A new age for selectivity?

The idea of using electric pulses for an improved selectivity in fishing gears rose already half a century ago, and although it's potential is being used only partially nowadays. As stated in Chapter 1, this has two main reasons: the technical challenge and the European legislation. Nevertheless, the number of electrotrawls has grown strongly in recent years, confirming the competitiveness and profitability of this technique compared to conventional gears. The wide-spread introduction promoted technical development and progress resulting in a new generation of pulse systems which are more user friendly and technically advanced and reliable. This may on its turn facilitate the development of new applications, both in beam trawls as in other fishing gears.

An obvious continuation of applying electric pulses is to look for an implementation in of the same electric stimuli in other fishing gears to increase their catch efficiency for sole. A first example of this is the implementation of the sole cramp pulse in twin rigs. This is being investigated by the ongoing 'Masterplan Duurzame Visserij - twinrigpuls' in the Netherlands where they aim for implementing the cramp stimulus for sole in front of the footrope of a twinrig trawl, to increase the catch efficiency for sole for this gear. Besides, the experiments with shrimp showed that shrimp demonstrate one upwards jump when exposed to a cramp pulse immediately followed by an escape response (Chapter 3 and 4). This reaction may enable fishermen to catch shrimp using a cramp stimulus, by catching the shrimp during their escape reaction. As the shrimp rebury quite quickly, the bobbin rope should immediately follow the conductor. Additionally, because the shrimp will have reached only limited height in the water column, a low position of the net seems recommended. Fishermen can also try to catch shrimp upon first contraction when they leave the seafloor, but this may require a footrope because the shrimp won't be high enough to use bobbins. However, the use of a cramp pulse, inducing an escape response in all fish, as well as the low position of the net or the use of a footrope would result in significant higher bycatches. Therefore, the use of a startle pulse is much more selective and recommended.

Exploring new electric pulses, by varying the pulse parameters may also offer new opportunities. Experiments showed that up to 15-25% of the sole leave the seafloor after being stimulated by the startle pulse applied in shrimp fishery (Polet *et al.*, 2005a; Desender *et al.*, 2015). This accord to the bycatch of commercial sole in electrotrawls targeting shrimp with this pulse, which is highest when sole is migrating to reproduce in spring (personal communication, Bart Verschueren). Besides, sole also show an escape response after being exposed to a short cramp stimulus in our studies (Chapter 5). Stewart (1975 & 1977) also reported jump-and-flight reactions in over 80% of the sole exposed to 20 Hz bursts of 1 s alternated with 1 s breaks. Although sole being buried in their natural habitat may react differently compared to the experimental studies, the escape response warrants further research as it might enable a different catch mechanism: sole can be caught during their flight or it can be used to herd sole by subsequent jump-and-flight reactions in front of the sweeps of an otter trawls.

Electric stimuli showed that they can be used to interact with or steer fish's behaviour. In fresh water, 'electric screens' are used to prevent fish from being drawn into an aquaduct or water inlets (Snyder, 2003a). This electric fencing is not possible in seawater, due to the high power demands and associated operation costs, but possible innovations are not restricted to increased catch efficiencies. Electric pulses may also be used in the post-catch in-trawl selectivity. This is typically done by using different mesh types or sizes, or inserting separator panels, grids or escape openings in the tail of the net. However, these net adaptions often lack effectivity because fish need to be activated by physical and visual stimuli (Glass & Wardlle, 1995; Kim & Whang, 2010; Herrmann *et al.*, 2015), which both have their limitations. The visual stimuli have the practical drawback that they often get clogged, while the visual stimuli can only be used in relatively shallow and clear water. Therefore, it is definitely worth investigating how this can be improved with electric stimuli. On one hand, they can be used to guide fish in a certain direction by repelling them with an unpleasant but non-immobilizing startle stimulus. A first possible application is to drive roundfish to large square mesh escape openings in the top of the net. A second is to stimulate sole's diving response, which may increase the (size) selectivity of a separator panel for plaice and sole. On the other hand, electric pulses may also inhibit reflexes or normal escape responses in fish. This was illustrated in the experiments with an electrified benthos release panel that used a cramp stimulus to prevent sole from diving through the panel (Chapter 8). The obtained results suggest that the loss of marketable sole may be avoided, which seems to be confirmed in the first 200 mm eBRP trials (data not shown). If the panel and stimulus are further optimized and implemented, it may be possible to release large quantities of stones, trash, invertebrates and undersized fish with almost no or minor losses of commercial catch. This would not only ameliorate the sorting process and catch quality, it may also directly amplify the fishermen's total allowable catches when the discard ban is fully applied. The promising results confirm that electric pulses are also useful to improve the selectivity of the trawl, which may trigger further innovations.

# General conclusion

Despite all studies on the effects of electrofishing done in recent years, some issues remain unanswered and sometimes new questions arose, offering new research opportunities. Aspiring to meet all of them, is not only infeasible, it may also be reckless. We will never be able to know, let alone to control, all processes that happen under the sea surface or inside trawls. Therefore, an important question is to what extent research should carry on and at what point one will have the courage to approve or reject a new trend. Since time and money are limited and answers need to be addressed quickly, research priorities have to be delineated to offer governmental institutions objective data to make decisions. Indeed, at present, a grey zone exists in which some fishermen are allowed to use electric stimulation and others not, which can be regarded as unfair competition in which some can and other cannot use a more profitable fishing gear.

Our results indicate that the net impact on adult invertebrates is in all probability smaller compared to conventional gears. In adult flatfish we could not demonstrate a direct side-effect. However, the major disadvantage of this electrogear remains the spinal injuries inflicted to Gadoid fish, of which our research indicates that they will continue to exist as long as cramp pulses are used. Therefore, this could damage the image of this alternative sustainable technique. Although some suggestions were made to prevent cod accessing high field strengths, it is uncertain how effective and practically feasible these are. Further laboratory research examining side-effects should therefore primarily focus on the extent and health effects of injuries in undersized Gadoidae. The obtained information should then be balanced against the advantages of electrotrawl such as lowered fish discards, reduced benthos by-catches and potentially lower trawl path mortality, less (intense) bottom impact and decreased fuel consumption, but more data from commercial fishing practice is required to do this properly.

So far, the results and general trend in research is definitely hopeful and it seems reasonable to state that they indicate a net-positive effect of electrotrawls for both the environment as the fishermen. However, further experimental studies including juvenile cod are warranted to give additional insight in the vulnerability of undersized Gadoidae, as well as the driving factors of the large inter-individual or inter-stock variability. Besides, shifting the focus more to the field is recommended because that may reveal unwanted in-situ interactions or possible long-term or indirect effects on commercial fish species, benthos and sensitive Gadoid fish that will be difficult to simulate in the laboratory. Therefore, management should also oblige a good monitoring of both conventional beam trawls and electrotrawls. This macro-approach will offer the best evidence of how a pulse fleet performs in relation to the conventional beam trawls. However, this may require a supra-national approach, since all except a few conventional Dutch beam-trawls targeting sole switched to a pulse gear (or stopped fishing). So far, this turn-over have not been reported to have adverse effects on fish stocks (personal communication with Kelle Moreau, ILVO, Belgium), but this should further be monitored carefully. Comparing the stock dynamics of Dutch fishing grounds for sole, where almost vessels switched to pulse gears in the past 5 years, with that of more Southern stocks where the beam trawl fleet was unchanged, can result in the first and very interesting indications to what extend the reduced by-catches, or possible side-effects on gadoid fish, affect the stocks and coming quota. Meanwhile, further innovation of the existing gears as well as further development of new applications improving the selectivity should be promoted.

# Recommendations for further research

Laboratory research

- Further investigation of the impact of pulse parameters on spinal injuries, with special focus on frequencies above 180 Hz.
- Determination of the decisive fish-specific parameters that promote spinal injuries, with special attention for differences in breading effects
- Exposing small isolated parts of the fish's body to gain insight in the physiological pathway(s?) leading to certain fish behaviour, in particular epileptiform seizures and cramp reactions provoking spinal injuries.
- Modelling the electric field/current inside (different tissues of the) fish body, in function of pulse parameters and the temperature, conductivity and electric field distribution (homogenous vs heterogenous).

Field research

- Side-effects of pulses: Catch comparisons with inliners targeting cod on electrotrawls using the cramp pulse to assess the injury rate in undersized cod.
- Catch efficiency: More elaborate catch comparisons to obtain correct and reliable values of differences in catch efficiencies and bycatch reductions per species.
- Bottom-impact: Impacts on different habitats comparing the mechanical impact of electrotrawls and conventional beam trawls on the substrate and associated invertebrate species
- Fishing effort: Analysing evolutions in landings, VMS data and fish stock assessments to examine different or altered fishing effort
- Selectivity: further experimental exploration of the potential of electric pulses for further innovation, e.g. the eBRP.

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# SUMMARY

# Summary

In traditional beam trawl fisheries, a gear comprising tickler chains, chain matrices or bobbin ropes is dragged over the seafloor to startle and catch flatfish or shrimp. These heavy fishing gears have well-known disadvantages such as seabed disturbance, fuel consumption and high by-catches. Pulse trawling is the most promising alternative for conventional beam trawling at this moment. In these electrotrawls, the mechanical stimulation by tickler chains, chain matrices or bobbins is (partly) replaced by electric stimulation, resulting in a less intensive drag and subsequently decreased seabed disturbance and fuel consumption. Additionally, the electric pulses generated by electrodes affect the target species more selectively, thus reducing by-catch. The nearly 100 vessels that have adopted this technique at this moment can be 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 Dover sole (*Solea solea* L.), by using a bipolar pulse of 60-80 Hz. This stimulus induces a cramp response in the sole's muscle, which makes it bend in a U-form and prevents it from escaping, resulting in increased catch efficiency. A minority of vessels target brown shrimp (*Crangon crangon* L.) by outfitting their boat with electrotrawls that produce a unipolar startle pulse of 5 Hz. These pulses force shrimp to flip their tail 5 times a second, which makes them jump out of the sediment in the water column. This allows the fishermen to catch them more selectively with less by-catch, also in clear water conditions. However, despite the promising opportunities, several concerns about negative effects of electric pulses on survival, behaviour and reproduction of target and non-target species need to be addressed (ICES recommendations, 2009), which led to the general aim of this thesis. **Chapter 1** elaborates on the history and development of the use of electric pulses in trawls, as well on possible side-effects, emphasizing the areas meriting further investigation. The consequent specific aims of the thesis may be found in **Chapter 2**, and include the investigation of possible side-effects of

electric pulses on adult invertebrates (**Chapter 3 & 4**), flatfish (**Chapter 5**) and roundfish (**Chapter 5, 6 & 7**) as well as testing new applications of pulse stimulation improving the selectivity (**Chapter 8**).

The first major gap in knowledge was the effect of electric pulses on marine benthic invertebrates. This was addressed in **Chapter 3**, presenting the results of experiments performed with brown shrimp (*Crangon crangon* L.) and ragworm (*Alita virens* S.) as model species for crustaceans and polychaetes, respectively. These animals were exposed to a homogenously distributed electric field with varying values of frequency, electric field strength, pulse polarity, pulse shape, pulse duration and exposure time to determine the range of safe pulse parameter settings and evaluate the effect of the pulses already being used on commercial electrotrawls. Behaviour during and shortly after exposure, 14 day (14-d) mortality rates, gross and histological examination were used to evaluate possible effects. No significant increase in mortality or injuries was encountered for either species within the range of pulse parameters tested. In contrast, examination of the hepatopancreas of shrimp exposed to the highest field strength revealed a significantly higher severity of an intranuclear bacilliform virus (IBV) infection. The obtained results hence were promising, but indirect effects, in particular on shrimp, as well as an increased impact of repetitive exposure under commercial conditions were still a major concern.

Therefore, brown shrimp were exposed 20 times in 4 days using commercial electrodes and pulse settings to catch shrimp (shrimp startle pulse) or sole (sole cramp pulse) and monitored for 14 days post first exposure (**Chapter 4**). Additionally to the previous experiment, also the size, egg loss, moulting and the degree of IBV infection were evaluated and compared between non-stressed non-exposed shrimp (control group 1) and shrimp exposed to electrodes without electric stimulus (control group 2) and as well as shrimp exposed to mechanical stimuli. In this study, no effect of electric stimulation on the degree of IBV infection was found. The survival of shrimp repetitively exposed to electric pulses did not significantly differ from those that were repetitively mechanically stimulated. However, the lowest survival was observed for the sole cramp pulse, and was significantly lower than in the second control group displaying the highest survival. On the other hand, the mechanically stimulated shrimp demonstrated the lowest percentage of moults compared to all other treatments, significantly lower than the second control group in which the highest percentage of moults was noted. Additionally, the mechanically stimulated shrimp that died during the experiment had a significantly larger size compared to the surviving individuals. Although negative impact of repetitive electric exposures on shrimp could not be ruled out, these results demonstrate that any impairing effects should be balanced against the harmful impact of the conventional trawls.

Despite being the major target species of beam trawls, no research investigating the effect of electric pulses on flatfish was reported so far. Dover sole (*Solea solea* L.), was therefore used as model species and exposed to over 40 different homogenously distributed electric fields with varying pulse parameters (**Chapter 5**). Fish behaviour during and shortly after exposure, 14-d post exposure mortality rates, as well as gross and histological examination was used to evaluate possible effects. Sole showed an escape response below a frequency of 20 Hz and a cramp reaction above 40 Hz, immediately followed by post-exposure escape behaviour. No mortality was observed and histological examination did not reveal any abnormalities, indicating the absence of irreversible lesions as a direct consequence of exposure to electric pulses in sole.

Atlantic cod (*Gadus morhua* L.) exposed in the same homogenous experimental set-up (**Chapter 5**), showed similar reactions during exposure. However, immediately after exposure to high electric loads, this gadoid round fish showed tonic-clonic epileptiform reactions. Moreover, one cod developed a spinal injury, which confirmed observations of cod with paravertebral bleedings in published laboratory and field research. Further research (**Chapter 6**) revealed that these epileptiform seizures were not observed when cod was exposed near electrotrawls' wire-shaped electrodes (heterogenous electric field) generating commercial cramp pulses, and may thus be promoted by the homogenous set-up with plate-shaped electrodes. The heterogenous set-up with cod aimed to investigate the variability in occurrence of electric-induced injuries in cod, by exposing wild cod and cultured cod from two different farms to the pulse used by electrotrawls targeting sole. Gross and radiographic examination revealed spinal injuries in 0-5% of fish when exposed near the electrodes. This contrasts with other studies showing incidences varying between 0 and 70% under the same experimental settings, demonstrating a fish-effect rather than a pulse (setting) effect. Analysis of the size, somatic weight, muscularity, number of vertebral bodies and vertebral mineral contents of cod of different origin did not reveal any (co-) decisive physiological nor morphological parameter for exhibiting vulnerability to electric pulses. However, some clues such as the impact of breeding-conditions definitely warrant further research.

Subsequently, we aimed to assess the vulnerability of another roundfish, sea bass (*Dicentrarchus labrax* L.), and compare its susceptibility for spinal injuries with that of gadoid roundfish such as cod and whiting (*Merlangius merlangus* L.) (**Chapter 7**). Therefore, sea bass were divided in 2 groups based on the size of the animals and exposed near commercial electrodes the same way as cod (Chapter 6). The behaviour during and after exposure was comparable to that of cod, but no epileptic seizures were induced in this heterogenous set-up. Further gross, radiographic and histologic examination did not demonstrate lesions, suggesting that bass is a less sensitive gadoid roundfish species. As a consequence, sea bass is not to be used as an alternative model species for all roundfish, and it is recommended to include other parameters besides anatomy of the musculature when examining the effect of electric pulses in future research.

The last study (**Chapter 8**) focussed on a possible new application of electric pulses, aiming in (further) improving the selectivity of beam and pulse trawl gears. Firstly, the conventional benthos release panels (BRP) were improved. These BRPs are known to release large amounts of benthos and debris which facilitate the sorting process as well as reduce the catch of undersized fish. However, unacceptable losses of commercial sole and damage to the BRP as a consequence of slack between the round net and square panel hampers a successful introduction in commercial beam trawl fisheries. To eliminate these drawbacks, the BRPs were inserted in square nets and the selectivity for BRPs square mesh size of 150 mm, 200 mm and 240 mm was assessed. Secondly, an electric cramp stimulus was implemented on the BRP to eliminate the loss of commercial sole. The first modification successfully eliminated the bag formation and subsequent damage while benthos and undersized fish were released in significant quantities. The results of the second innovation suggest that sole larger than 25 cm was retained, without negatively affecting the release of benthos and most undersized commercial fish. Although further research using smaller mesh sizes or optimized electric stimuli to achieve retention of all commercial sole is warranted, this study clearly demonstrates the promising potential of electric stimuli for further innovation.

Finally, an overall discussion of the scientific results and future research perspectives are provided in **Chapter 9**. The laboratory findings and implications for the field are reviewed, subsequently focusing on the estimated total impact of electrotrawls and elaborating on further innovations that may be created.

# SAMENVATTING

# Samenvatting

De conventionele boomkorvisserij gebruikt zware wekkers, kettingmatten en klossen om platvis en garnaal te vangen. Deze vistuigen worden over de bodem gesleept waardoor ze een grote bodemimpact en een groot brandstofverbruik kennen. Bovendien zijn ze weinig selectief waardoor ze veel ongewenste bijvangst hebben. Pulsvissen is op dit moment het best beschikbare alternatief. In de zogenaamde pulskor wordt de mechanische stimulering door middel van kettingen of klossen grotendeels vervangen door elektrische stimulatie met behulp van elektrodes die voor het net gehangen worden. Deze produceren elektrische pulsen die specifiek de doelsoort stimuleren waardoor er minder andere soorten bijgevangen worden. Bovendien gaan door het wegvallen van de kettingen ook de bodemimpact en het brandstofverbruik sterk achteruit, wat bijgevolg resulteert in een verminderde impact op het milieu en lagere kosten voor de visser. Bijna 100 boomkorren maken op dit moment al gebruik van elektrische stimulatie, waarbij er twee verschillende type boten en pulsen gebruikt worden naargelang de doelsoort. De eerste en meest voorkomende is gericht op platvis, in het bijzonder op tong (*Solea solea* L.), en gebruikt daarvoor een kramppuls van 60-80 Hz. Blootstelling aan deze puls induceert een kramp reactie in de spieren van de tong, waardoor deze in een U-vorm buigt en niet meer kan ontsnappen. Daarnaast jaagt een minderheid van de pulsvissers op grijze garnaal (*Crangon crangon* L.) door gebruik te maken van een schrikpuls van 5 Hz die ervoor zorgt dat de garnalen 5 keer per seconde gaan opspringen en uit het zand in de waterkolom terecht komen. Hierdoor kan de visser de garnalen uit het water vangen met minder bijvangst en kan hij bovendien ook garnaal vangen bij helder water. Ondanks deze veelbelovende mogelijkheden, rijzen er echter nog heel wat vragen bij de mogelijke neveneffecten van deze nieuwe technologie op de overleving, het gedrag en de voortplanting van de bodemdieren die er aan worden blootgesteld. **Hoofdstuk 1** leidt deze studie in met een overzicht van de geschiedenis en de ontwikkeling van elektrische pulsen in sleepnetten om vervolgens de mogelijke neveneffecten en hiaten in de kennis te beschrijven. Op basis daarvan werden de specifieke doelstellingen van deze thesis bepaald, die omschreven staan in **Hoofdstuk 2**. Het hoofddoel was het afbakenen van de veilige zone voor het gebruik van elektrische pulsen zonder ernstige neveneffecten op ongewervelden (**Hoofdstuk 3 & 4**), platvis (**Hoofdstuk 5**) en rondvis (**Hoofdstuk 5, 6 & 7**), waarna ook nieuwe toepassingen van elektrische pulsen bestudeerd werden die tot een verdere verbetering van de selectiviteit kunnen leiden (**Hoofdstuk 8**).

Het eerste grote hiaat in de kennis was het effect van elektrische pulsen op mariene bodeminvertebraten. In **Hoofdstuk 3** worden de resultaten voorgesteld van de proeven met grijze garnaal (*Crangon crangon* L.) en de zager (*Alita virens* S.) die als modelsoort fungeerden voor respectievelijk de crustacea en de polychaeten. Deze dieren werden blootgesteld in een homogeen elektrisch veld met variabele frequentie, veldsterkte, pulstype, pulsvorm, pulsduur en blootstellingstijd om ,enerzijds de veilige bovengrens te bepalen van elke puls parameter, en anderzijds mogelijke neveneffecten te evalueren van de pulsen die reeds gebruikt worden in commerciële pulskotters. Daarbij werden zowel het gedrag tijdens als na de blootstelling, de 14 daagse overleving en macroscopische en microscopische letsels geëvalueerd door garnaal en zager bloot te stellen tussen plaatelektroden. Voor geen van beide modelsoorten werd een significante toename van sterfte of letsels vastgesteld. Histologische analyse van de hepatopancreas van de garnaal toonde wel een toename van intranucleaire baciliforme virussen (IBV) bij garnalen die waren blootgesteld aan de hoogste veldsterkte. Het uitblijven van letsels en sterfte bij zager en garnaal was bemoedigend, maar de mogelijke indirecte effecten op garnaal en de impact van herhaaldelijke blootstelling onder commerciële omstandigheden bleven evenwel een bezorgdheid.

Daarom werd een volgend experiment op poten gezet waarbij garnaal 20 keer werd blootgesteld in 4 dagen (**Hoofdstuk 4**). Ditmaal werden draadvormige elektroden van een commerciële pulskor gebruikt met bijhorende elektrische pulsen: een schrikpuls voor garnaal en een kramppuls voor tong. Naast de overleving, letsels en de aanwezigheid van IBV virussen werd eveneens de mate waarin garnalen hun eieren verloren en vervelden gemonitord. Deze data werd vergeleken met: een groep die niet gestrest was, een groep die was blootgesteld aan de elektroden zonder dat een puls werd gegeven en een groep die werd blootgesteld aan mechanische stimuli, zijnde een voorbijkomende ketting van een conventionele boomkor. Ditmaal werd er geen effect van de elektrische stimulus op de ernst van de IBV infectie waargenomen. De overleving van de elektrisch gestimuleerde garnalen week ook niet significant af van deze die mechanisch werden gestimuleerd. Het laagste overlevingspercentage werd vastgesteld voor garnalen blootgesteld aan de tongpuls, en deze was significant lager dan de groep met het hoogste overlevingspercentage, namelijk deze die blootgesteld werden aan de elektroden zonder puls. Anderzijds vertoonde de mechanisch gestimuleerde groep significant minder vervellingen dan deze controlegroep, en bleek bovendien dat de dode mechanisch gestimuleerde garnalen significant groter waren dan de overlevenden. Ondanks het feit dat negatieve effecten dus niet konden worden uitgesloten, geven deze data wel aan dat elk neveneffect van de pulskor moet afgewogen worden tegen mechanische schade die door conventionele boomkorren wordt veroorzaakt.

Verrassend genoeg werd er tot dusver geen onderzoek gedaan naar mogelijke schade door elektrische pulsen bij platvis. De belangrijkste doelsoort, tong werd daarom gebruikt als modelsoort en blootgesteld aan meer dan 40 verschillende pulsen met variërende pulsparameters in een homogeen elektrisch veld (**Hoofdstuk 5**). Het gedrag van tong tijdens en na blootstelling, de overleving tot 14 dagen na blootstelling en uitwendige letsels werden gemonitord. Daarnaast werden er ook RX-opnames gemaakt om mogelijke fracturen vast te stellen en werden de inwendige organen histologisch onderzocht op afwijkingen. Tong vertoonde tijdens blootstelling een vluchtreactie indien pulsen met een frequentie van 20 Hz of lager werden gebruikt, terwijl pulsen met een frequentie hoger dan 40 Hz resulteerden in een krampreactie. Na blootstelling was er meestal een vluchtrespons. Blootstelling aan elektrische pulsen veroorzaakte echter geen sterfte, noch uitwendige of microscopische letsels aan de organen en het skelet. Dit wijst erop dat tong een blootstelling aan elektrische pulsen goed kan weerstaan zonder directe of onomkeerbare letsels.

Kabeljauw (*Gadus morhua* L.) blootgesteld in een identieke homogene opstelling (**Hoofdstuk 5**), vertoonde gelijkaardige reacties als tong tijdens de blootstelling. Onmiddellijk na blootstelling aan zeer sterke pulsen vertoonden zij evenwel epileptiforme aanvallen. Bovendien vertoonde één kabeljauw ook een wervelluxatie en bloedingen rond de wervelkolom, wat in het verleden ook al door andere onderzoekers gerapporteerd werd. Verder onderzoek toonde aan dat deze epileptiforme aanvallen niet optraden indien de kabeljauwen naast draadvormige elektroden werden blootgesteld aan de kramppuls voor tong zoals dat op commerciële pulskotters gebeurt (**Hoofdstuk 6**). Dit kan er op wijzen dat de homogene elektrische velden, voortgebracht door plaatvormige elektroden zoals in de eerste studie, deze epileptiforme aanvallen promoten en versterken. Deze tweede, heterogene, studie (Hoofdstuk 6) had als doel de variabele gevoeligheid voor elektrisch geïnduceerde letsels bij kabeljauw te onderzoeken. Daartoe werden wilde en gekweekte kabeljauw van verschillende kwekerijen vlak naast de elektrodes blootgesteld aan de kramppuls voor tong. Autopsie en RX-foto’s brachten breuken aan het licht bij 0-5% van de dieren, wat sterk afweek van de 0-70% die gerapporteerd werd in een voorgaande studie die gebruik maakte van exact dezelfde experimentele opstelling. Dit toonde aan dat de variatie slechts gedeeltelijk aan de manier van blootstellen kan worden toegeschreven en dat vis-gerelateerde parameters een doorslaggevende rol spelen. Daarom werden de lengte, het somatisch gewicht, de gespierdheid, het aantal wervels en het mineraalgehalte van de verschillende groepen van kabeljauw vergeleken. Deze analyse kon geen doorslaggevende fysiologische of morfologische parameter aantonen waardoor sommige dieren gevoeliger zouden zijn aan elektrisch geïnduceerde letsels. Het werd echter wel gesuggereerd dat andere factoren, zoals de kweekomstandigheden, een belangrijke rol zouden kunnen gespeeld hebben.

Vervolgens werd de gevoeligheid voor elektrische pulsen van een andere rondvis, zeebaars (*Dicentrarchus labrax L*), vergeleken met deze van gadoide rondvis zoals kabeljauw en wijting (*Merlangius merlangus* L.) (**Hoofdstuk 7**). Daartoe werden 2 groepen zeebaars van verschillende grootte blootgesteld op identiek dezelfde wijze als kabeljauw tijdens het experiment beschreven in Hoofdstuk 6. Net zoals kabeljauw vertoonden alle zeebaars een krampreactie tijdens blootstelling, meestal gevolgd door een korte vluchtreactie onmiddellijk erna. Er werden evenwel geen epileptiforme aanvallen vastgesteld. Autopsie, RX-analyse en histologisch onderzoek brachten geen enkel letsel aan het licht, wat lijkt te suggereren dat deze rondvis minder gevoelig is dan gadoide rondvis en dus niet bruikbaar is als alternatief modelorganisme in verder onderzoek. Daarnaast toont deze studie aan dat verder onderzoek naar de gevoeligheid van rondvis voor elektrisch geïnduceerde letsels naast de anatomie van de spieren, ook moet focussen op andere morfologische en fysiologische parameters.

De laatste studie (**Hoofdstuk 8**) concentreerde zich op mogelijke nieuwe toepassingen van elektrische pulsen teneinde de selectiviteit van de boom- of pulskor verder te verbeteren. Daartoe werden eerst de bestaande benthos ontsnappingspanelen (BRP) verbeterd. Deze BRP’s zijn een grootmazig paneel in de onderkant van het net, waar benthos en afval doorheen kunnen vallen vooraleer deze in de kuil terecht komen. Dit resulteert in een efficiëntere verwerking en betere kwaliteit van de vis. Bovendien vangt een net met BRP ook minder ondermaatse vis. Het nadeel van deze BRP’s is enerzijds dat ook een deel van de commerciële tong ontsnapt en anderzijds dat er zakvorming en schade optreedt als het rechthoekig paneel in een rond net wordt geplaatst. Om dit op te lossen werd het BRP in deze studie in een vierkant net geplaatst, waarna de selectiviteit voor verschillende BRP maaswijdtes (150 mm, 200 mm en 240 mm) werd getest. Deze aanpassing elimineerde succesvol de zakvorming en de daarmee gepaard gaande schade terwijl er nog steeds grote hoeveelheden aan benthos en ondermaatse vis geloosd werd. In een 2$^e$ fase werd een elektrisch veld aangebracht op het paneel, waardoor er geen tong groter dan 25 cm meer leek te ontsnappen. Het verlies aan benthos of ondermaatse vis werd hierdoor echter niet verminderd. Verder onderzoek moet nagaan of kleinere paneelmaaswijdtes en/of een optimalisatie van de elektrische puls het verlies van commerciële tong helemaal kan voorkomen. Deze resultaten tonen nogmaals duidelijk het veelbelovend karakter en potentiële innovatie van elektrische stimuli aan.

De thesis eindigt met een algemene discussie over de wetenschappelijke resultaten en toekomstige onderzoeksmogelijkheden (**Hoofdstuk 9**). Hierin worden de bevindingen, die in experimentele omstandigheden werden bekomen, overlopen en de worden implicaties daarvan voor de situatie op zee besproken. Tot slot wordt de totale impact van pulsvissers ingeschat en worden mogelijke nieuwe innovaties en opties voor verder onderzoek gesuggereerd.

# EXECUTIVE SUMMARY

The important improve in bycatch, bottom impact and fuel consumption of electrotrawls compared to conventional beam trawls, as well as the fast acceptance by the sector indicate that the use of electric pulses in fisheries is very promising. However, a good knowledge of possible threats is indispensable to avoid problems in the long term. Therefore, an overview of all studies performed by the end of 2015 examining the effect of electrotrawls and the electric pulses used is given below. To evaluate the effects of electric pulses on marine organisms, laboratory studies have included several model species for invertebrates, roundfish, flatfish, electro-sensitive fish and non-commercial fish species to cover the broad range of animals that are commercially exposed to pulses by electrotrawls. The results of such studies, listed in the table above, illustrate that the worst effects have been observed in gadoid fish like cod and whiting. These species can develop spinal injuries and haemorrhages during exposure to electric pulses. Additionally, reduced survival have been observed in one 1 larval stage of cod. Such unwanted side-effects were not found in other roundfish such as seabass, elaborate experiments including a broad range of pulses with sole, studies focusing on electro-sensitive animals such as dogfish or in exposure studies with by-catch species of shrimp fisheries. Moreover, extensive exposures of invertebrates such as ragworm and shrimp could not evidence a larger impact of electric stimulation compared to conventional mechanical stimulation.

Although these findings are reassuring, lab experiments cannot cover all possible effects. Indeed, it is impossible to account for all environmental variables of electric exposures in laboratory studies, fish's behaviour in the wild, possible inter-species and even inter-individual variation as observed for cod, the interaction with moving electrodes and the gear,… Moreover, the impact on the animal and ecosystem is not only determined by the short electric exposure, but may be affected on the longer term by indirect effects of reduced bycatches and discards, less intense sea-floor impact, altered fishing efforts and spatio-temporal shifts to new fishing grounds. Although cod for example may have a reduced survival or recruitment when exposed to electric pulses, the reduced catches and the increased chances on escape and survival resulting from the smaller catch volumes and lower towing speeds may result in a net positive effect on cod.

It is therefore necessary to focus more on the impact on a larger scale and longer term, i.e. the impact/performance of an electrotrawl vessel on one hand, and that of an electrotrawl fleet on the other. The first can be done through extensive catch comparisons, which would give us insight in the altered catches of both target and non-target species on different substrates an during different seasons. An in-depth monitoring by scientists would also allow us to do an elaborate and quick assessment of the real extent of injuries inflicted to gadoid species and to assess the impact on species that were not included in laboratory tests. Secondly, a thoroughly analysis of the (change in) landings of electrotrawls in relation to the (new) fishing grounds and changes in stock assessments for these areas would definitely give valuable indications of how the switch from mechanical to electric stimulation affect certain species and stocks and what the real difference in net-impact on the environment may be.

A good impartial legislation and reinforcement defining the maximal allowed pulse outputs (peak voltage and duty cycle) is crucial for several reasons. First, the comparison and interpretation of catch data, landings or stock trends over a couple of years is less instructive if pulse settings are unknown or increase over time. Second, this regulation should prevent fishermen and constructors of increasing the strength of the pulses used, because although they might result in higher catch efficiencies for the target species, side-effects also show a positive correlation with pulse intensity, as shown for cod. Third, because most effect studies done so far were limited to the pulse (gear) settings currently used. Allowing the industry to use stronger pulses implies that the presented results are no longer representative. To tackle the latter issue, further experiments and/or modelling possible side-effects in function of the pulse settings used are warranted.

In conclusion, there is no evidence that the pulse settings tested had major side-effects in the model-species studied, except for gadoid species. Although this should further be evidenced by (long term) date of the field, the limited number of side-effects discovered seems to be exceeded by the reduced bycatches and bottom contact. This suggests that the total impact of electrotrawls on the marine ecosystem, especially those targeting shrimp, may be smaller than that of conventional beam trawls. However, until all above concerns are refuted, caution remains warranted.

Synthesis of research done on pulse fishing (end 2015) and their results. The data is divided in 3 categories according the pulse parameter and/or gear that was used during the experiments. The electric pulse settings used during the experiments are listed in the second column. Exposures to electric pulses in effect-studies were carried out using commercial electrodes, expect when mentioned differently (plate electrodes). References to studies included in this PhD research are indicated in bold in the last column.

| | | Study of effect of | | | Species or electrotrawl | Results | References |
|---|---|---|---|---|---|---|---|
| Electrotrawl targetting shrimp | 5 Hz PDC pulses of 500 µs; 60 V; 0,6 m electrode distance | electric startle pulses on | early life stages | roundfish | cod | reduced survival in 1 out of 8 stadia exposed (3, 4 and 1 stages of egg, larval and juveniles respectively; plate electrodes), no injuries after 1 and 16 or more days post exposure | 1 |
| | | | | flatfish | sole | no increased mortality, no injuries after 14 days between plate electrodes | 2 |
| | | | adult animals | roundfish | cod | no injuries or mortality after 1 day and 14 days | 6, **7** |
| | | | | flatfish | sole | no mortality, no injuries after 1 day and 14 days | 6, **7** |
| | | | | | plaice | 2 small microscopic haemorrhages but no macroscopic injuries or mortality after 1 day | 6 |
| | | | | electro-sensitive fish | dogfish | no mortality or injuries, no interference with electro-sensitive organs in terms of pred detection | 8 |
| | | | | non-commercial fish | bull-rout | no mortality or injuries after 1 day | 6 |
| | | | | invertebrates | bullhead | no mortality or injuries after 1 day | 6 |
| | | | | | shrimp | no increased mortality after 15 s exposure between plate electrodes | 3 |
| | | | | | | no mortality, no injuries 14 days after exposure between plate electrodes | **5** |
| | | | | | | no increased mortality, no injuries, no size-specific effects, no release of eggs 14 days after 20 exposurs in 4 days | **4** |
| | | | | | ragworm | no mortality, no injuries 14 days after exposure between plate electrodes | **5** |
| | | gear | bottom contact | | HA31 | 60% reduction for 12 bobbins in straight bobbinrope compared to a conventional round bobbinrope with 36 bobbins | 18 |
| | | | bottom impact | | O82, HA31 | sampling done during Benthis campaign 2015. Results are not published yet. | |
| | | | catch comparison | | O191, TX25, SD33 | by-catch reduced with 15-65% depending on rigging | 19 |
| | | | | | HA31 | 0-16% more commercial shrimp (depending on saison), 19-33% less non-commercial shrimp, 50-76% less by-catch | 18 |
| | | | drag & fuel consumption | | HA31 | 22,8% more power required to trawl a conventional gear, no data on fuel consumption | 18 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Electrotrawl targetting sole | 40-80 Hz PBC or PAC pulses of <500 µs; 50-60V; 0,32-0,42 m electrode distance | electric cramp pulses on | early life stages | roundfish | cod | no mortality or injuries observed in juveniles (12-16 cm) exposed near the electrodes | 14 |
| | | | | | | no studies done so far on eggs and larvae because these vessels are usually not fishing in shallow and nursery waters | |
| | | | adult animals | roundfish | cod | ± 10% of cod find with spinal injuries in the field | 12, **13** |
| | | | | | | No mortality or injuries when exposed above or outside the electrodes | 14 |
| | | | | | | 0-70% when exposed near the electrodes with strong inter-animal variability in sensitivity | **7**, 14, **15** |
| | | | | | | epileptiform seizures observed in adult cod when exposed to cramp pulse for sole between plate electrodes | **7** |
| | | | | | whiting | 2% injuries observed in catch comparison | 12 |
| | | | | | seabass | no mortality or injuries 14 days after exposure in near field | **16** |
| | | | | flatfish | sole | no mortality or injuries 14 days after exposure between plate electrodes | **7** |
| | | | | | dab | no mortality or injuries after 5 days | 11 |
| | | | | electro-sensitive fish | dogfish | no mortality or injuries, no damage observed on electro-sensitive organs | 8, 17 |
| | | | | non-commercial fish | | no studies done so far | |
| | | | | invertebrates | shrimp | no mortality or injuries 14 days after exposure between plate electrodes, increase in viral bodies at 200 V $m^{-1}$ | **5** |
| | | | | | | No increased mortality, no injuries and no release of eggs compared to mechanical stimulation 14 d after 20 exposures | **4** |
| | | | | | ragworm | no mortality, no injuries after 14 days | **5** |
| | | | | | other species | exploratory study with 19 species of molluscs, echinoderm, crustaceans and polychaetes: no increased mortality after 3 weeks | 9 |
| | | | | | | exploratory study with 6 species at different distances from electrodes: variable and contradictory results after 2 weeks | 10 |
| | | | | | | => Both exploratory studies conclude 'no increased impact compared to mechanical stimulation' | 9, 10 |
| | | gear | bottom impact | | SCH18 | no differences in quantity of sediment mobilized; tickler-chain penetrates the seabed deeper than electrotrawl | 20, 21 |
| | | | | | TX43 | sampling of 12 m beam trawls done during Benthis campaign 2014. Results are not published yet. | |
| | | | catch comparison | | TX36, TX68 | 62% fewer benthos discards, 44% fewer fish discards, 21% decrease in marketable sole based on 33-48 hauls in one week | 12 |
| | | | | | fleet | 16% and 42% reduction in number of starfish and crabs respectively, non-significant effect on fish discards and | 22 |
| | | | | | | 10-20% increase in marketable sole based on a year-round sampling program including 50% of the electrotrawl fleet | 22 |
| | | | drag & fuel consumption | | TX36, TX68 | 50% fewer drag resulting in 37-49% fuel consumption | 12 |
| | | | | | fleet | 14% lower towing speed and 0-40% lower fuel consumption based on models | 23 |
| Beyond current pulse settings | | | electric pulses on adult animals | roundfish | cod | increasing the frequency to 180 Hz may reduce the number of spinal injuries; lower field strengths result in less injuries | 14 |
| | | | | | | doubling the pulse amplitude to 120 V induces epileptiform seizures and results in substantial higher rate of spinal injuries | **15** |
| | | | | flatfish | sole | no mortality or injuries 14 days after exposure to 47 different pulses with varaying and maximized pulse parameters between plate electrodes | **7** |
| | | | | invertebrates | shrimp | no mortality or injuries 14 days after 2 s exposures to pulses of 200 Hz or 1000 µs pulse duration (60 Hz) (plate electrodes) | **5** |
| | | | | | ragworm | no mortality or injuries 14 days after 2 s exposures to pulses with varaying and maximized pulse parameters (plate electrodes) | **5** |

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# CURRICULUM VITAE
# &
# BIBLIOGRAPHY

# Curriculum Vitae

Maarten Soetaert werd geboren op 30 april 1988 te Ninove. Na het beëindigen van het secundair onderwijs aan het Sint-Aloysius college in Ninove begon hij in 2006 met de studie bio-ingenieur aan de Universiteit Gent. In 2010 ging hij op Erasmus uitwisseling naar Montpellier en in 2011 behaalde hij zijn diploma in de specialisatie milieutechnologie. Zijn afstudeerwerk 'Biodegradation of micropollutants after biokathodic dehalogenation' werd bekroond met de Arcelor Mittal-Indaver milieuprijs.

Na het afstuderen startte hij zijn doctoraatsonderzoek, ondersteund door een IWT-mandaat, bij de vakgroepen Morfologie en Pathologie, Bacteriologie en Pluimveeziekten van de Universiteit Gent in samenwerking het met Instituut voor Landbouw en Visserij Onderzoek. Zijn onderzoek was gericht op het in kaart brengen van de effecten van elektrische pulsen op marine organismen en mogelijke nieuwe toepassingen van elektrische pulsen binnen de visserij. Verder kreeg hij de mogelijkheid om in het voorjaar van 2013 gedurende drie maanden proeven uit te voeren met kabeljauw bij de Noorse onderzoeksinstituten Nofima in Tromsø en IMR in Austevoll.

Hij behaalde in 2013 het certificaat proefleider en in 2014 volgde hij met succes de BTC infocyclus ontwikkelingssamenwerking. In 2015 vervolledigde hij het trainingsprogramma van de Doctoral School of Life Sciences and Medicine van de Universiteit Gent. Hij gaf presentaties op verschillende internationale congressen en was gemandateerd lid van ICES SG ELECTRA en WG ELECTRA. Hij is auteur en coauteur van meerdere wetenschappelijke publicaties in nationale en internationale wetenschappelijke tijdschriften en trad tevens op als reviewer.

# International publications

De Gusseme, B., Hennebel, T., Vanhaecke, L., **Soetaert, M.**, Verstraete, W. and Boon, N. (2011). Biogenic palladium enhances diatrizoate removal from hospital wastewater in a microbial electrolysis cell. Environmental Science and Technology (IF 5.48 , Q1).

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Devriese L. and Soetaert, M. 2013. Afname zweren op Vlaamse schar: Piek veroorzaakt door wier? Visserijnieuws 34, 1p

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Devriese, L., Soetaert, M., Bekaert, K., Desender, M., Chiers, K., Decostere, A. and Polet, H. 2015. Huidzweren bij vissen in het Belgisch deel van de Noordzee, Trends in prevalentie en exploratie van mogelijke oorzaken. ILVO-mededeling 188, ISSN 1784-3197, 24 p.

Soetaert, M., Verschueren, B. and Vanderperren, E. 2015. ILVO test eBRP in vierkant net. Visserijnieuws 11, 3 p and Rederscentrale maart, 3 p.

Co-author of: VLIZ (2014). Beleidsinformerende Nota: De effecten van pulsvisserij door Nederlandse vaartuigen in Belgische wateren – een synthese van het Vlaams wetenschappelijk onderzoek. VLIZ Beleidsinformerende nota's BIN 2014_001. Oostende. 22 pp.

# Oral presentations

## *For scientists*

'The impact of electrotrawls on marine organisms' on VLIZ Young Marine Scientists' Day' (KHBO, Brugge, 15 February 2013)

'The impact of electrical pulses on benthic invertebrates' on the ICES-symposium 'Effects of fishing on benthic fauna and habitats' (Tromsø, Norway, 16-19 July 2014)

'The electrified Benthos Release Panel (eBRP)' on the ICES-group of Fisheries Technology and Fish Behaviour (FTFB) (Lissabon, Portugal, 4-7 May

2015)'Planned experiments of PhD research' on the yearly workshop of the ICES study group on electric fishing (SG ELECTRA) (Orléans, France, 21-22 April 2012)

'PhD research: first results' on the yearly workshop of the ICES study group on electric fishing (SG ELECTRA) (Ostend, Belgium, 23-25 October 2013)

'The impact of electric pulses on benthic species' on the yearly workshop of the ICES working group on electric fishing (WG ELECTRA) (Ostend, Belgium, 22-24 October 2014)

'Overview of results obtained during PhD research' on the yearly workshop of the ICES working group on electric fishing (WG ELECTRA) (Ijmuiden, The Netherlands, 10-12 November 2015)

## *For fishermen*

'Effecten van electrische pulsen op zager, garnaal, tong en kabeljauw' on the Puls Informatie-/discussiedag organized by the Stuurgroep Pulsvisserij (5th of March, 2012)

'Pulsvissen en zijn effecten op mariene dieren' on the Infomoment pulsvissen (ILVO, Ostend, 6th of November 2013)

'Neveneffecten van electrische pulsen' on SVT themadag garnalenvisserij (Emmeloord, 23th of May, 2014)

## *For laymen*

'Electrotrawls: impact on marine organisms' on Duurzame Visweek (Ghent, 7th of November 2013)

'Duurzame vis' on de smaakboot (vistournee/week van de smaak, 13-23 November 2014)

# Poster presentations

‘Flatfish fishery: impact and challenges‘ on VLIZ Young Marine Scientists' Day' (KHBO, Brugge, 24 February 2012)

‘Flatfish fishery: impact and challenges‘ on FMV 2nd Scientific Meeting (Faculté de Médecine vétérinaire, Liège, 19 oktober 2012)

‘Possible application of electrical pulses for a more selective fishery’ on the first MARES-congress ‘Marine ecosystem health and conservation’ (17-21 November 2014, Olhao, Portugal)

# Scientific awards

Best thesis: 1st laureate of the Arcelor Mittal milieuprijs 2011 mmv Indaver with master thesis ‘Biodegradation of micropollutants after biokathodic dehalogenation’.

Best oral presentation: VLIZ Young Marine Scientists’ Day 2013 with ‘The impact of electrotrawls on marine organisms’

Best movie: of the Marine@Ugent Video Contest 2014 with ‘Jumping is not a crime, it’s a way of fishing’: https://www.youtube.com/watch?v=pwOnzMaazTA

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Cover: Swimming sole, *Solea solea* L., Unknown

Chapter 1:Beam trawl on the North Sea, Karl Van Ginderdeuren

Chapter 2: Sunset in Erstfjord, Tromsø, Maarten Soetaert

Chapter 3: *Alitta Virens* S., Alexander Semenov

Chapter 4: *Crangon crangon* L., Ron Offermans

Chapter 5: *Solea solea* L., Ron Offermans

Chapter 6: Sacrificing of *Gadus morhua* L., Maarten Soetaert

Chapter 7: Autopsy and RX of *Dicentrarchus Labrax* L., Maarten Soetaert

Chapter 8: Benthos Release Panel in trawl, Maarten Soetaert

Chapter 9: Hauling of the catch, ILVO Techniek

Summaries: View from Vengsøya, Maarten Soetaert

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