Effects of electrical stimulation on marine organisms

Pim G. Boute

Propositions

1. Effects of electrical stimulation on marine animals during pulse trawling are negligible compared to the mechanical effects of bottom-trawl gears.
   (this thesis)

2. Spinal injuries in Atlantic cod (*Gadus morhua*) caused by electrical stimulation are acceptable collateral damage of pulse trawling.
   (this thesis)

3. Carapace morphology of boxfishes (Ostraciidae) has evolved to promote manoeuvrability.
   (Boute, P.G., Van Wassenbergh, S., Stamhuis, E.J. 2020. Modulating yaw with an unstable rigid body and a course-stabilizing or steering caudal fin in the yellow boxfish (*Ostracion cubicus*). *Royal Society Open Science*, 7: 200129)

4. Climate change will lead to low biomass and productivity in surface waters of the Mediterranean Sea.
   (van de Poll, W.H., Boute, P.G., Rozema, P.D., Buma, A.G.J., Kulk, G., Rijkenberg, M.J.A. 2015. Sea surface temperature control of taxon specific phytoplankton production along an oligotrophic gradient in the Mediterranean Sea. *Marine Chemistry*, 177: 536–544)

5. Pushing for animal-free experiments will restrict biomedical research and environmental impact studies.

6. Monarchy is undemocratic.

Propositions belonging to the thesis, entitled

Effects of electrical stimulation on marine organisms

Pim G. Boute
Wageningen, 25 April 2022

Effects of electrical stimulation on marine organisms

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Chapter 1

General introduction

Marine capture fisheries play an important role in global food supply and provide livelihoods for millions of people (Kent, 1997; Béné *et al.*, 2016; FAO, 2020). Key in food security is the sustainable exploitation of wild aquatic stocks with minimal environmental impact (Garcia and Rosenberg, 2010; McClanahan *et al.*, 2015; FAO, 2020). Divergent fishing methods and gears have been developed to harvest aquatic resources, depending on the target species and habitat (Jennings *et al.*, 2001). Environmental impact reduction is particularly warranted in bottom trawling (Lindeboom and de Groot, 1998). This technique involves dragging nets over the seafloor to capture demersal and benthic organisms and is characterised by relatively poor selectivity, large disturbance of the benthic ecosystem, and high fuel consumption (de Groot, 1984). For example, bottom trawling for common sole (*Solea solea*) in the North Sea conventionally involves towing so-called tickler chains in front of the net to mechanically stimulate the fish from the sediment (Rijnsdorp *et al.*, 2008; Eigaard *et al.*, 2016). An alternative method is to replace the tickler chains by electrode arrays which generate pulsed electric fields (Soetaert *et al.*, 2015a). This electrical stimulus induces involuntary muscle contractions which immobilise fishes and enables subsequent capture (Soetaert *et al.*, 2015a). Besides positive effects such as reduced fuel use and benthic disturbance (van Marlen *et al.*, 2014; Depestele *et al.*, 2019; Tiano *et al.*, 2019; Poos *et al.*, 2020), pulse trawling also has negative effects such as inducing spinal fractures in Atlantic cod (*Gadus morhua*) (van Marlen *et al.*, 2014; de Haan *et al.*, 2016; Soetaert *et al.*, 2016a). These negative aspects of electrical stimulation have raised concerns regarding other effects of pulse trawling on marine organisms. Therefore, in this thesis, we studied some effects of electrical pulse stimulation on benthic invertebrates and fishes and explore the ecological implications of electrotrawling.

1.1 Marine capture fisheries: sustainable harvest with minimal environmental impact

Global marine catches from wild stocks have increased rapidly since the middle of the 20th century and have stabilised at about 90 million metric tonnes reported annually since the late 1980s (Garcia and Grainger, 2005; Pauly and Zeller, 2016; Watson and Tidd, 2018; FAO, 2020). Overexploitation remains a major problem worldwide, driving fish stocks to low abundance and affecting non-target species, habitats, and even entire ecosystems (Pauly and Christensen, 1995; FAO, 2020; Palomares *et al.*, 2020; Sumaila and Tai, 2020). Traditionally, fisheries management has mainly focused on the harvest of fish populations and maximizing catches by e.g. reduction of fishing effort (Hjort, 1914, 1926; Auster *et al.*, 1996; Hilborn and Ovando, 2014; Froese *et al.*, 2018). Recently, however, focus of fisheries management and research has shifted towards assessing and minimising the environmental impact of fishing activities into an ecosystem-based approach (Goñi, 1998; Jennings and Kaiser, 1998; Pikitch *et al.*, 2004; Palumbi *et al.*, 2009; Rijnsdorp *et al.*, 2016). Demersal fisheries account for about 30% of the global catches, have a large spatial footprint, and include some of the most damaging harvest techniques such as bottom trawling (Halpern *et al.*, 2008, 2015; Eigaard *et al.*, 2017; Amoroso *et al.*, 2018; Watson and Tidd, 2018; Pitcher *et al.*, 2022).

Bottom trawling is a fishing method which involves dragging a net or similar collection device (i.e. the trawl) over the seafloor to catch demersal and benthic organisms including fish, shellfish, and crustaceans. Consequently, bottom trawl gears affect the seafloor structure, benthic communities, and the environment in various ways: (i) the gears disturb and homogenise the seabed texture (Paschen *et al.*, 2000; Puig *et al.*, 2012; Eigaard *et al.*, 2016); (ii) biogeochemical processes are altered due to e.g. resuspension of fine sediments into the water (Bradshaw *et al.*, 2021; Breimann *et al.*, 2022); (iii) bottom trawls damage benthic habitats and cause direct mortality on organisms that are not retained by the gear (Clark *et al.*, 2016; Hiddink *et al.*, 2017; Sciberras *et al.*, 2018); (iv) bottom trawls are not selective and therefore generate a substantial amount of bycatch, which is partly discarded e.g., because species are commercially uninteresting or below the minimum landings size (Uhlmann *et al.*, 2014; Kennelly and Broadhurst, 2021); (v) bottom trawling can alter food web interactions and ecosystems e.g., because some species are more vulnerable to disturbances whilst others recover faster (Heath, 2005; van Denderen *et al.*, 2013); (vi) bottom trawling usually involves vessels with powerful engines to tow the generally heavy gears, which results in high fuel consumption and carbon dioxide emissions (Parker and Tyedmers, 2015; Parker *et al.*, 2018). Hence, environmental effects of bottom-trawl fisheries are a major concern and urgently require impact reduction by improving selectivity, reducing benthic disturbance, and reducing operational power requirement (Jennings and Kaiser, 1998; Suuronen *et al.*, 2012; McConnaughey *et al.*, 2020). In other words, we need to integrate the sustainable harvest of the target species with a minimal environmental impact.

1.2 Bottom-trawl fishery targeting common sole in the North Sea

Demersal fishing has been ongoing in the North Sea since the 13th century (Collins, 1889; de Groot, 1984; Hovart, 1985; Thurstan *et al.*, 2010; Posthumus and Rijnsdorp, 2016; Lescrauwaet *et al.*, 2018). Large-scale bottom trawling in the mixed-fishery for common sole with so-called beam trawls and tickler chains started in the 1960s when powerful engines became available (Rijnsdorp and Millner, 1996; Rijnsdorp *et al.*, 2008). These tickler chains are dragged over the seafloor to drive the fish into the net. In subsequent years, fishers invested in larger vessels to increase gear size, towing speed, and number of tickler chains. This increasing fishing capacity sparked concerns about the potential environmental impact in the following decades (Lindeboom and de Groot, 1998; Linnane *et al.*, 2000).

The bottom-dwelling common sole is notoriously difficult to catch. These slender and flexible fish require relatively small cod-end mesh sizes to prevent specimens slipping through. In addition, this species buries when sensing danger, making it harder to catch. Finally, the fish are nocturnal and generally remain buried during the day (Kruuk, 1963). Consequently, beam trawls have a number of design requirements that enable capture of common sole: (i) a mechanism to keep the gear on the seafloor; (ii) a mechanism to keep the mouth of the net open in horizontal and vertical directions; (iii) a net which guides the animals that enter the mouth inwards to (iv) the cod-end with suitable mesh size where the specimens are collected until hauling; (v) a stimulation mechanism to chase the fish from the seafloor over the ground rope into the net. In beam trawls, the beam keeps the net open and is attached to shoes that slide over the seafloor (**Figure 1.1A**, **E** and **1.2A**, **B**). A more recent modification is the SumWing, where the beam is replaced by a hydrofoil with a single slider which results in reduced fuel consumption (**Figure 1.1C** and **1.2C**) (van Marlen *et al.*, 2009a; Rijnsdorp *et al.*, 2021a). A net is placed above the trawl mouth to enclose animals that enter. Tickler chains or a chain mat are used as mechanical stimulus whereby the latter is used on rough fishing grounds and prevents entrance of large stones in the trawl. This mechanical stimulus makes beam trawls amongst the most damaging fishing gears worldwide.

Beam trawls are effective at catching common sole but at a high environmental cost. Although developed in a quest for increasing catch rates and reducing of fuel consumption, replacing tickler chains with electrode arrays comes with promising environmental advantages (Soetaert *et al.*, 2015a). These so-called pulse trawls use pulsed electric fields as stimulation mechanism to drive common sole from the seafloor (**Figure 1.1B**, **D**, **F** and **1.2C**). In short, the pulsed electric fields between the electrode arrays elicit a muscle cramp, which inhibits the fish's escape ability. In particular, the stimulus makes common sole bend in U-shape and easy to catch (see e.g. **Chapter 2** and **4**). For the advantages of electrotrawling with pulses over conventional tickler-chain trawling, see introductions of e.g. **Chapter 3**, **4**, and **6**.

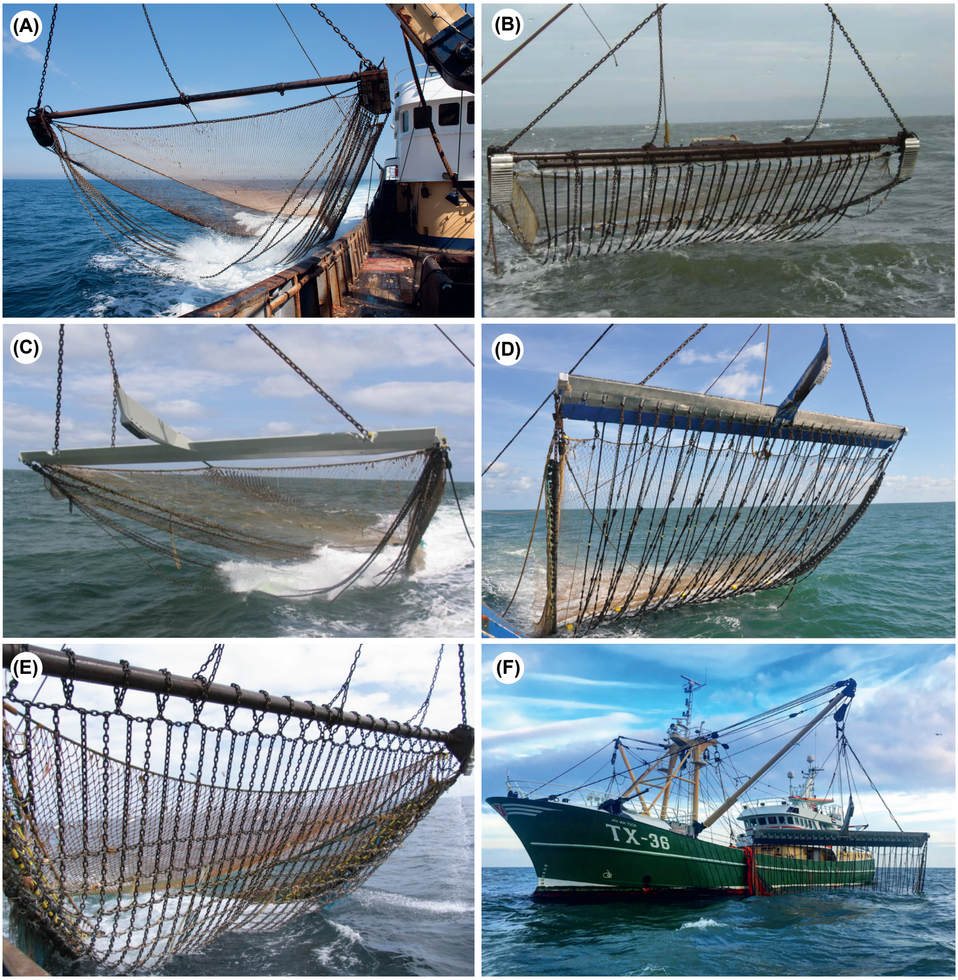


Figure 1.1. Bottom-trawl gears used to target common sole in the North Sea. **(A)** Conventional tickler-chain beam trawl with ticklers attached to the shoes and net (latter not visible: underwater). **(B)** Pulse gear of Delmeco Group B.V. (previously: Verburg Holland) with electrodes running from the beam to the ground rope (latter not visible: underwater). **(C)** SumWing tickler-chain trawl where the beam and two sliders are replaced by a hydrofoil with a single slider. **(D)** PulseWing of HFK Engineering B.V. where the pulse modules are incorporated in the SumWing. **(E)** Conventional chain-mat gear. **(F)** Example of how the aforementioned gears – in this case a PulseWing – are attached to the fishing vessel, with one gear on port side and one on starboard (latter not visible). All photographs show gears with a 12 m beam width towed by a cutter with a ≤1,467 kW engine and hull length of 30–45.99 m, which are not allowed to fish in territorial waters. Smaller, so-called Eurocutter vessels (≤221 kW engine power; maximum length of 23.99 m) are allowed to fish the 12 nautical mile zone with beams of ≤4.5 m, but sometimes also fish with 7–8 m gears outside territorial waters (Rijnsdorp *et al.*, 2021a). Photograph credits: **(A)** Albert Romkes; **(B)** Wageningen Marine Research; **(C)** Flanders Marine Institute photo gallery / Fisheries and Aquatic Production; **(D)** Wageningen Marine Research; **(E)** Flanders Research Institute for Agriculture, Fisheries and Food; **(F)** Jan van der Vis via Nederlandse Vissersbond.

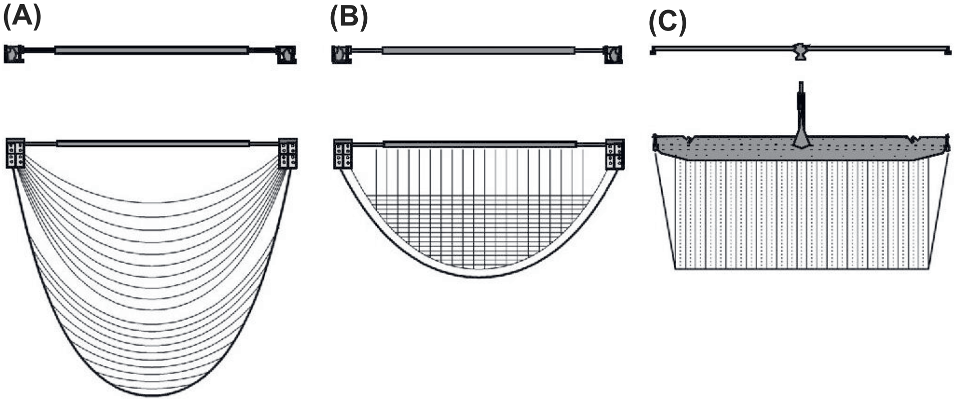


Figure 1.2. Schematic drawings of the three 12 m beam-trawl types used in the common-sole fishery in the North Sea, photographs shown in **Figure 1.1**. Top and bottom panels show the frontal and bottom view respectively and dimensions between gears are scaled. **(A)** Conventional beam trawl with tickler chains attached to the shoes (shoe ticklers) and ground rope (net ticklers). **(B)** Conventional chain-mat beam trawl with an orthogonal chain framework attached to the beam and second ground rope (thin curved line between the shoes). **(C)** PulseWing trawl with a rectangular ground rope, electrode arrays (solid lines), and tension relief cords (dotted lines). These cords run between the electrode arrays from the PulseWing to the ground rope and release tension on the electrode arrays and maintain the shape of the ground rope. Tickler chains and electrode arrays can be combined with a beam or a wing as shown in **Figure 1.1**. Different ground rope configurations for the trawl types and the netting material are not shown. Figure is modified from Rijnsdorp *et al.* (2021a).

1.2.1 A short history on marine electrotrawling

According to de Groot and Boonstra (1974), the likely earliest record of thinking about applying electricity to capture marine organisms was in 1765 by Job Baster when he wrote (in old Dutch):

> "Zoude nu de electriciteit, wier schokken zoo veel overeenkomst heeft met die de sidderaal veroorzaakt, op de garnaat (garnaal) geen uitwerking doen? My dunkt, het is waardig, zulks te onderzoeken."
>
> (translation to modern English: "Would electricity, which shocks are so similar to those produced by the electric eel, have no effects on shrimp? In my opinion, it would be worthwhile to investigate this.")

A more concrete development by Isham Baggs in 1863 was patenting the idea to use electricity from batteries for, amongst others, the capture of fish (Baggs, 1863). It was not until the 20th century, however, that attention shifted to investigate the physiology and behaviour of marine organisms in response to electrical stimulation in shrimps but also echinoderms, flatfish, round fish, and whales (Scheminzky, 1931; Houston Jr., 1949; Groody *et al.*, 1952; Bary, 1956; Highman, 1956; Halsband, 1958; Dragesund and Leivestad, 1959; Kessler, 1965; Blancheteau, 1971; Le Men, 1971; Klima, 1972;

Stewart, 1973a, 1979; Seidel and Vanselous, 1976; Sternin *et al.*, 1976; Danulitė and Malkavichus, 1976; Delanghe and Vanden Broucke, 1978). In addition, electrofishing in highly-conductive seawater posed technical challenges including a corrosive environment and large power demands (Stewart, 1973b; ICES, 1975; Sternin *et al.*, 1976; Anon, 1977; Malkiavichus, 1977). Different electrical waveforms were used to study responses of animals. The majority of these studies focused on the application of freshwater electrofishing knowledge to attract, immobilise, or stun aquatic organisms with electric fields (i.e. galvanotaxis/electrotaxis and electronarcosis). For details on freshwater electrofishing, which developments started earlier and progressed faster than in marine electrofishing, see e.g. Vibert (1963, 1967), Sternin *et al.* (1976), Bohlin *et al.* (1989), Cowx (1990), Cowx and Lamarque (1990), Snyder (2003), Reynolds and Kolz (2012), Beaumont (2016), and Reynolds and Dean (2020).

Simultaneously with the rise of the beam-trawl fishery in the North Sea during the 1960s, fishing trials with electrified trawls started for common sole – and other marine fish species and invertebrates – in the Netherlands (de Groot and Boonstra, 1970; Boonstra and de Groot, 1974; Agricola, 1985), Belgium (Vanden Broucke, 1972; Vanden Broucke and Vanhee, 1977; Vanden Broucke and Delanghe, 1979), United Kingdom (Stewart, 1974, 1975a, 1975b, 1976), West Germany (Schärfe, 1965; Horn, 1976, 1977, 1986), United States of America (McRae and French Jr., 1965; Pease, 1967; Pease and Seidel, 1967; Wathne and Holt, 1967; Seidel, 1969; Seidel and Klima, 1974; Seidel and Watson Jr., 1978), India (Namboodiri *et al.*, 1977), and the Union of Soviet Socialist Republics (Sternin *et al.*, 1976; ICES, 2010, 2011). The main aims were to reduce fuel consumption and increase catch efficiency and selectivity compared to conventional catch methods. In a few cases increased survival opportunities of discards and improved catch quality were additional objectives (van Marlen, 1997). None of these gears left the experimental design and test phase due to costs involved, malfunctioning, and vulnerability of the technology, although commercialisation attempts were made in the Netherlands (van Marlen, 1997). Globally, most research ceased by the end of the 1980s for various reasons including: (i) fear for overfishing as harvest control rules were being implemented but not yet strictly followed; (ii) less incentive to reduce fuel consumption because the 1970s energy crisis had ended; (iii) deaths of involved scientists.

In the 1990s, research into electrotrawling for brown shrimp (*Crangon crangon*) and common sole was continued in Belgium and the Netherlands respectively. Research and development on common sole was mainly done by a private company. In the meantime, commercial pulse trawling for shrimp had started in the East China Sea (Zhou, 1999 as cited in Yu *et al.*, 2007). This fishery peaked in the late 1990s at about 3,500 pulse trawlers out of 10,000 trawlers in total, but collapsed due to lack of regulations leading to overfishing (Yu *et al.*, 2007). The shrimp pulse technique caught new attention by some Belgium fishers and scientists, which had made visits to China (van Marlen, 1997; Polet, 1999; Polet *et al.*, 2005a; pers. comm. Hans Polet). At the end of the 1990s, as interests increased to reduce adverse environmental impact of fishing gears, van Marlen (1997) and Linnane *et al.* (2000) posed the idea of minimising seafloor contact with electrotrawls. They underscored the potential of electrofishing, providing that issues regarding fisher safety and equipment robustness are solved. Furthermore, fishers should receive economic compensation to finance high investment costs.

Halfway the 2000s, rising oil prices were an incentive to start trials on research and commercial vessels in Belgium (main focus on brown shrimp) and the Netherlands (main focus on common sole). As of 2007, pulse trawlers were allowed to fish in the southern North Sea International-Council-for-the-Exploration-of-the-Sea (ICES) areas IVb and IVc (Council of the European Union, 2006, 2007), which overlaps with the spatial distribution of common sole (Heessen *et al.*, 2015) and brown shrimp (ICES, 2019; Schulte *et al.*, 2020). This sparked a new wave of research focusing – again – on fuel consumption, catch efficiency, and selectivity (Polet *et al.*, 2005a, 2005b; van Marlen *et al.*, 2006, 2014; Steenbergen and van Marlen, 2009; Verschueren *et al.*, 2019, 2012; Taal and Klok, 2014; Soetaert *et al.*, 2016b; Turenhout *et al.*, 2016; Rijnsdorp *et al.*, 2021b) as well as economic investment theory (Jensen *et al.*, 2019). In addition, environmental aspects were studied including discard survival (van Marlen *et al.*, 2005; van der Reijden *et al.*, 2017; Schram and Molenaar, 2018), potential negative effects of electrical stimulation on marine organisms (van Marlen *et al.*, 2001, 2007, 2009b; Smaal and Brummelhuis, 2005; de Haan *et al.*, 2009b, 2009a, 2011, 2013, 2015, 2016; Soetaert *et al.*, 2015b, 2016a, 2016c, 2016d, 2018; Desender *et al.*, 2018, 2016, 2017a, 2017b; Schram and Molenaar, 2019; Bergman and Meesters, 2020), physical benthic disturbance (Depestele *et al.*, 2016, 2019), spatio-temporal fleet distribution (Sys *et al.*, 2016; Turenhout *et al.*, 2016), effects on a larger, ecological scale (Stepputtis *et al.*, 2014; Teal *et al.*, 2014; Ford *et al.*, 2019; Vansteenbrugge *et al.*, 2020), and, in some instances, how to minimise any negative effects by e.g. choosing other electrical waveform parameters (Soetaert *et al.*, 2015b, 2016c). Some of the aforementioned studies have only appeared in grey literature, or have later been published as peer-reviewed papers which results in overlapping publications. In addition, some of these studies focused on the 5 Hz pulse frequency brown-shrimp technique whilst others studied the 30–45 Hz common sole pulse waveform. Simultaneously, electrofishing techniques for endobenthic razor clams (*Ensis* spp.) have been developed in Ireland and Scotland (Breen *et al.*, 2011; Murray *et al.*, 2016; Fox *et al.*, 2019), albeit with another waveform and collection occurs mostly by divers instead of nets or dredges (see **Chapter 2**). Risk for overfishing the common sole stock was less of an issue as fisheries management was well-established

(ICES, 2020, 2021), but no quota exists for brown shrimp. For further details on marine electrofishing history, see van Marlen (1997), Linnane *et al.* (2000), van Marlen *et al.* (2010), Soetaert *et al.* (2015a), Haasnoot *et al.* (2016), and ICES (2018).

1.2.2 Knowledge gaps and open questions

During the diverse research history of marine electrotrawling, the emphasis has mainly been on overcoming technical issues in making a reliable fishing gear and increasing catch efficiency at minimal fuel costs. The leading incentive was the potential of increased economic benefit. Relatively late, research focus shifted to studying the potential negative impact of electrical stimulation on marine organisms. In this thesis, we focus on the effects of the flatfish pulse waveform on marine organisms.

Some effects of flatfish pulse trawling on marine biota have been addressed in aforementioned research. However, many questions have remained unanswered (Quirijns *et al.*, 2013, 2015, 2018; Kraan *et al.*, 2015; Steins *et al.*, 2017; Kraan and Schadeberg, 2018; Bremner *et al.*, 2019). Extrapolating findings from studies before the 2000s is problematic because either electrical stimulation details are missing, or the waveforms used were different from what has been used in pulse trawling for common sole thereafter. More recently, studies found spinal fractures and internal haemorrhages in Atlantic cod caught with the flatfish pulse waveform (van Marlen *et al.*, 2014; de Haan *et al.*, 2016; Soetaert *et al.*, 2016a, 2016c). This aggravated concerns as internal injuries in fish had likewise been found in freshwater electrofishing studies (Snyder, 2003).

In this thesis, we attempt to fill some of the knowledge gaps regarding the effects of electrical stimulation on marine organisms. Specifically, we are interested in the effect of electrical stimulation on: (i) behaviour and survival of benthic invertebrates; (ii) behavioural response thresholds of electroreceptive and non-electroreceptive fish species; (iii) the internal-injury incidence in fish caught by pulse trawls. In the latter case, we also quantify injuries in catches from pulse trawls with the electrical stimulus turned off and conventional tickler-chain gear to compare findings.

1.3 Aims and outline of this thesis

With this thesis, we aim to achieve two goals. First, we want to contribute to the mechanistic understanding regarding the effects of pulsed electric fields on marine organisms by studying responses of benthic invertebrates and fishes to electrical stimulation. Second, we attempt to translate our findings into insights useful to assess the impact of flatfish electrotrawling on marine organisms. The obtained insights are used in an interdisciplinary, collaborative research consortium, the "Impact Assessment Pulse-trawl Fishery" (IAPF) project, shared between the Experimental Zoology Group of Wageningen University & Research (NL), the Department of Estuarine & Delta Systems of the Royal Netherlands Institute for Sea Research (NL), the Fishery and Aquatic Production Department of the Flanders Research Institute for Agriculture, Fisheries, and Food (BE), and Wageningen Marine Research of Wageningen University & Research (NL) (**Figure 1.3**). This thesis is embedded in the IAPF project. In this project, we aim for a deeper understanding of the effects of electrical stimulation used by pulse trawlers on the marine ecosystem. Hereto, IAPF integrates the insights on marine organisms (this thesis) with the effect of pulse trawls on the functioning and biogeochemistry of benthic ecosystems as well as characteristics and dynamics of the fishing fleet and population effects at the North Sea level into an impact assessment on a transition of the tickler-chain fleet to a pulse-trawl fleet regarding potential adverse ecosystem effects (Rijnsdorp *et al.*, 2020).

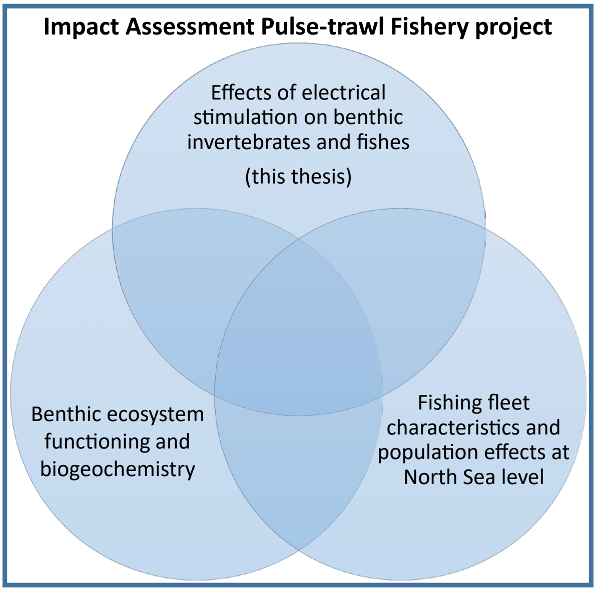


Figure 1.3. Schematic overview of the "Impact Assessment Pulse-trawl Fishery" project highlighting the three major research themes that were used for an impact assessment.

In this thesis, we used a multidisciplinary approach to acquire understanding on how marine organisms respond to the electrical stimulus, which is required to scale up potential effects to ecosystem level and to achieve our research goals. We combined laboratory experiments, optical animal tracking, statistical modelling, numerical modelling, X-radiography, dissection, and data collection in the field to study and quantify electric fields, animal behaviour, response thresholds, and internal injuries in a range of species.

In **Chapter 2**, we review the marine electrotrawling field, describe physiological responses of organisms exposed to electrical stimulation, and outline waveform characteristics. We identify a lack of consistency in the description of electrical parameters in marine electrofishing laboratory and field research. For example, Depestele *et al.* (2019) used a 60 Hz Pulsed Bipolar Current waveform, which was identical to the 30 Hz bipolar waveform described by de Haan *et al.* (2016). This creates unnecessary confusion, hampers comparison of study results, obstructs identification of possible causes for deviating findings, and potentially undermines regulatory documentation of pulse trawlers needed for control and enforcement by the authorities. We offer recommendations for better communication standards in electrofishing and pulse trawling in particular. We propose to standardise terminology and descriptions of electrical waveform parameters, experimental designs, and environmental parameters. Finally, we hope that our work promotes collaboration with the freshwater electrofishing research community as these fields are currently, in our opinion, insufficiently connected. We think that, despite the differences, both study areas could benefit from the exchange of ideas, challenges, and unanswered questions regarding the use of electrical stimulation to capture organisms, both for monitoring and commercial purposes.

In the subsequent chapters, we address aforementioned knowledge gaps and research goals (**Figure 1.4**). Here, we focus on a range of marine invertebrate and fish species. The North Sea is inhabited by about 1200 invertebrate and about 201 fish species (Fransz *et al.*, 1991; Künitzer *et al.*, 1992; Holtmann *et al.*, 1996; Rogers *et al.*, 1998; Krause *et al.*, 2003; Rees *et al.*, 2007; Witbaard *et al.*, 2013; Heessen *et al.*, 2015; Bos *et al.*, 2016). Their distribution and abundance is highly heterogeneous, depending on e.g. habitat, daily, seasonal or annual migration patterns related to feeding and spawning, egg and larval survival, and fishing intensity. This variety makes it unfortunately impossible (or at least highly challenging, time-consuming, and expensive) to study all species and potential effects. A choice for species was, therefore, made for several scientific and practical reasons:

i. Species should be relevant for studying pulse-trawl impact. Therefore, we excluded pelagic invertebrate and fish species as the electrical stimulus is generally only active when the gear is on the seafloor (pers. comm. Harmen Klein Woolthuis of HFK Engineering B.V.; see **Chapter 7**) and focus on species that inhabit the relatively shallow southern North Sea.
ii. Some species have previously been studied in electrofishing research, albeit in some instances published in grey literature only, thus providing sufficient

reference data to discuss obtained results and, where possible, extrapolate findings (Smaal and Brummelhuis, 2005; de Haan *et al.*, 2009a, 2015; van Marlen *et al.*, 2014; de Haan *et al.*, 2016; van Marlen *et al.*, 2009b; Desender *et al.*, 2016, 2017b; Soetaert *et al.*, 2016c, 2016a, 2018).

iii. Differences in body plans – i.e. different phyla (Valentine, 2006) – allow an assessment of different neuromuscular and musculoskeletal systems as we chose benthic invertebrate species belonging to the phyla Annelida, Arthropoda (subphylum Crustacea), Echinodermata, and Mollusca.
iv. Differences in e.g. body shape (round fish and flatfish) and sensory systems (electroreceptive and non-electroreceptive) enable us to assess potential effects of morphology and anatomy on behaviour and injury incidence.
v. Living specimens should be feasible to collect, transport, handle, and house in captive conditions.
vi. In case of dead animals, species should be present in catches of commercial fishing operations and relatively abundant to collect a sufficiently large sample size.

Apart from a choice in species, we also chose different experimental designs for our experiments with living animals to tackle our research questions. We used either an exposure in a homogenous electric field with plate-shaped electrodes (**Chapter 3**) or offered varying electric field strengths in a heterogeneous field created by rod-shaped electrodes (**Chapter 4**). The former exposure type was used to create a worst-case-scenario exposure to consistently-high field strengths by placing the animals in the same orientation (Soetaert *et al.*, 2015b). For the latter exposure type, changes in location (position and orientation) of the animal with respect to the electrode pair as well as pulse amplitude variation created a range of field strength exposures. These data were subsequently used to quantify a response threshold as function of field strength which were translated to distances around commercial electrodes. In both chapters, we used a pulse exposure duration of 3 s, which is about twice as long as during field conditions, either to increase the worst-case effect or to better observe the response. Apart from exposure duration and electric field strength, other electrical pulse settings (i.e. waveform type, pulse shape, frequency, pulse width, and duty cycle) were always similar to the waveforms used by commercial pulse trawlers to translate findings to field conditions.

In **Chapter 3**, we studied effects of electrical pulse stimulation on the behaviour and survival of six marine benthic invertebrate species, i.e. common starfish (*Asterias rubens*), serpent star (*Ophiura ophiura*), common whelk (*Buccinum undatum*), sea mouse (*Aphrodita aculeata*), common hermit crab (*Pagurus bernhardus*), and flying crab (*Liocarcinus holsatus*) (**Figure 1.4**). In particular, we aimed to quantify species-specific locomotion behaviour that would indicate prolonged changes related to increased predation risk, namely indirect survival due to e.g., impaired locomotor performance. We also scored acute behaviour during exposure and subsequent recovery period to reveal potential different response mechanisms between species. Animals were placed, one at the time, in an experimental arena where we created a worst-case-scenario exposure. We quantified the specimen's locomotion-behaviour responses before, during, and after electrical stimulation and compared these to a non-exposed control group. In addition, we monitored survival for 14 days post-exposure for both treatment groups to assess potential long-term effects of electrical stimulation.

Next, we studied behavioural responses of five fish species as function of electric field strength in **Chapter 4**. Here, we estimated behavioural response thresholds of the electroreceptive small-spotted catshark (*Scyliorhinus canicula*) and thornback ray (*Raja clavata*), and the non-electroreceptive common sole, European seabass (*Dicentrarchus labrax*), and turbot (*Scophthalmus maximus*) for electric field strengths (**Figure 1.4**). Using camera recordings from above a circular tank with electrode pairs creating a heterogeneous electric field, we assessed behavioural responses for different pulse amplitudes and for different fish positions relative to the stimulating electrodes during a staircase procedure. Hereafter, we used a numerical simulation of the electric field in our experimental setup, verified with *in situ* measurements, to determine the field strength at the animal's location for each stimulus. With a receiver-operating characteristic analysis, we quantified response thresholds of individuals. Subsequently, we calculated species-specific thresholds and compared electroreceptive and non-electroreceptive species. Lastly, we related the response thresholds to field strengths around a commercial electrode array pair computed with a numerical simulation, hence providing an estimate for response distances with respect to the fishing gear.

In addition to behavioural responses and survival, concerns exists on fish-species susceptibility to vertebral-column injuries and internal haemorrhages. In **Chapter 5**, we studied the previously hypothesised susceptibility of Gadidae (Soetaert *et al*., 2018) by quantifying internal injuries in whiting (*Merlangius merlangus*) catches from pulse trawls with and without electrical stimulation, and conventional beam trawls with tickler chains (**Figure 1.4**). Spinal injuries and haemorrhages were visualised with X-ray photography and dissection respectively, scored on a severity scale, and quantified on the anteroposterior fish axis. We assessed spinal injury and haemorrhage probability as function of fish length for each severity and catch method. Finally, we examined the co-occurrence of spinal injuries and haemorrhages. We used the above quantitative assessment of injuries to shed light on the causes of injuries in whiting caught by commercial fishing vessels and discuss the ecological consequences.

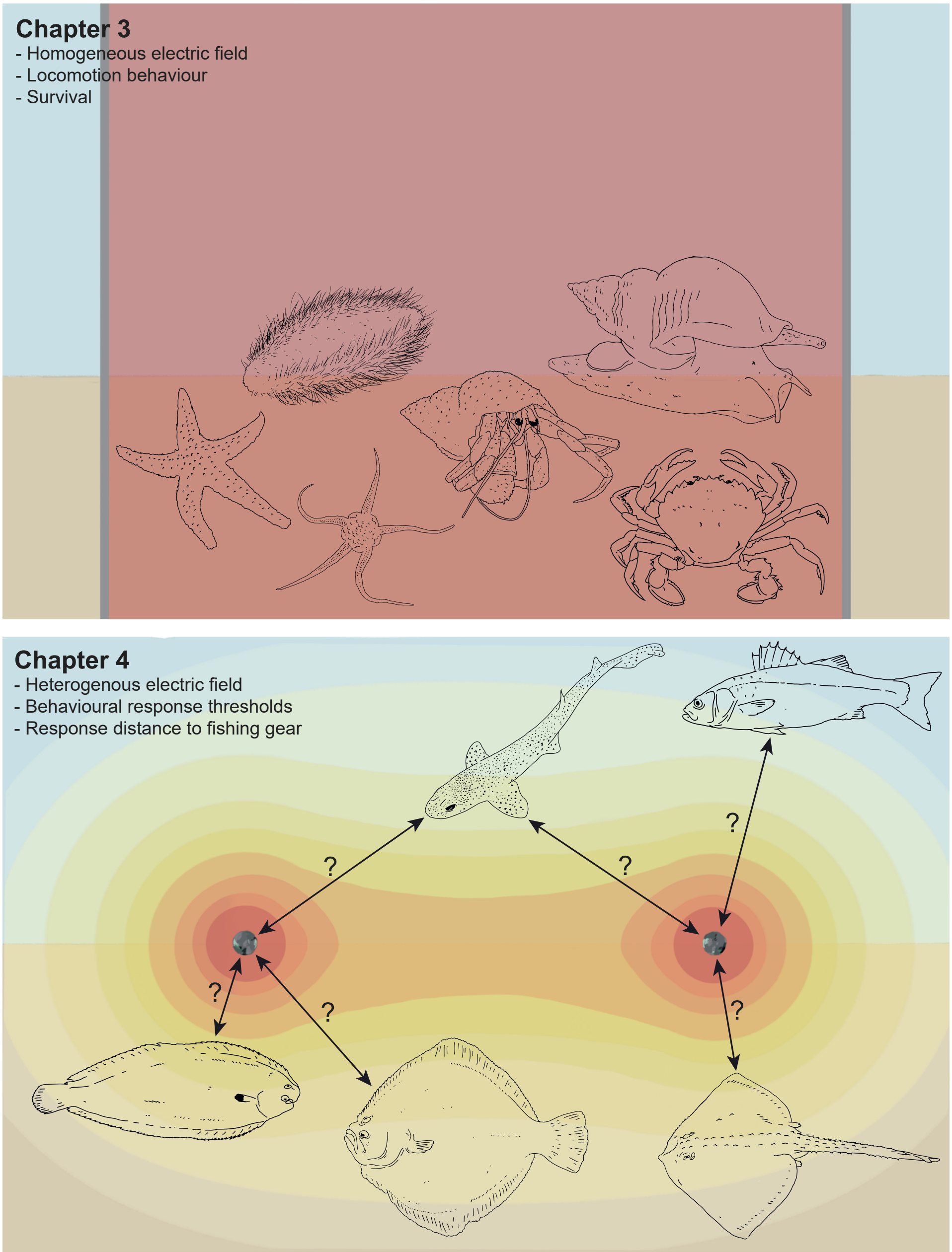


Figure 1.4. Schematic overview of the experimental research chapters. All panels show a cross-section through an electrode pair (grey/metallic) where hotter colours denote higher electric field strengths. Components are not drawn to scale and depicted animals do not affect the electric field. In **Chapter 3**, we studied how marine benthic invertebrates respond to electrical pulse stimulation by quantifying their behaviour and post-exposure survival. In **Chapter 4**, we investigated behavioural response thresholds for electric field strength in electroreceptive and non-electroreceptive fishes and related these to the electric field around the electrode arrays of a pulse trawl. Illustrations by Kazuma Eekman.

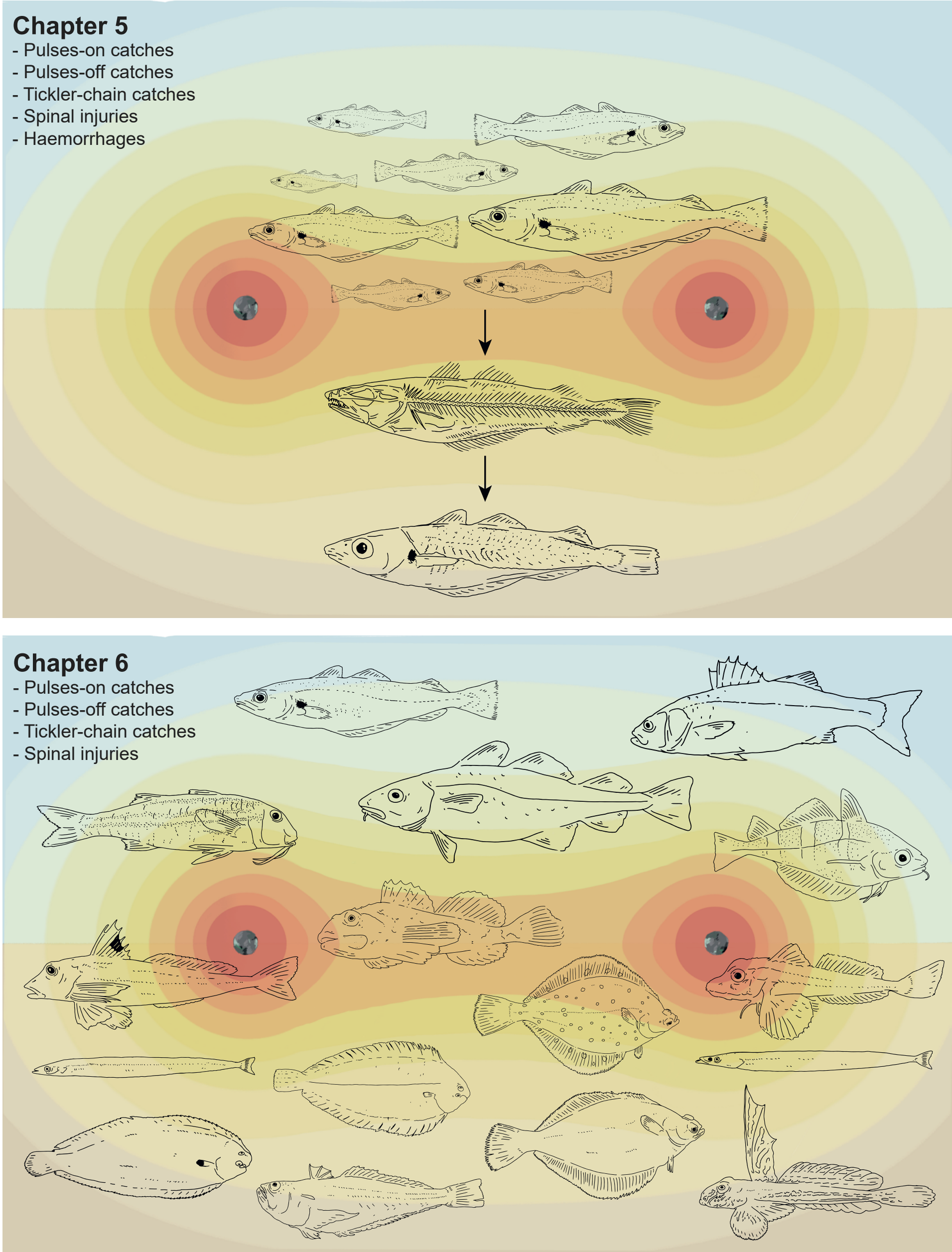
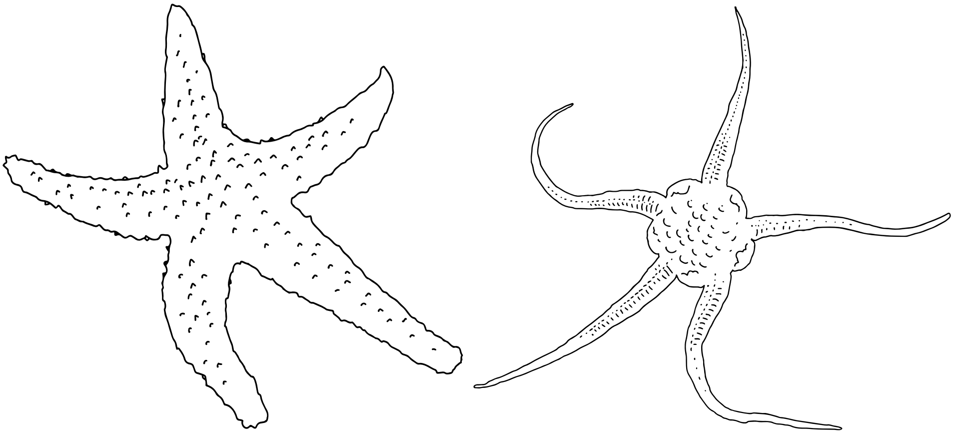
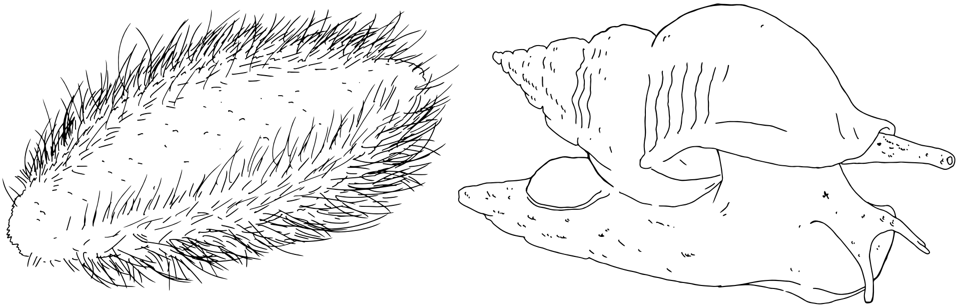


Figure 1.4. (continuation). In **Chapter 5**, we examined internal injuries in whiting caught by pulse trawlers with electrical pulses turned on or off and compared these to samples from conventional tickler-chain trawlers. In **Chapter 6**, we assessed spinal injuries in a range of fish species that were sampled from commercial catches and discussed whether injuries were more likely caused by electrical or mechanical stimulation.



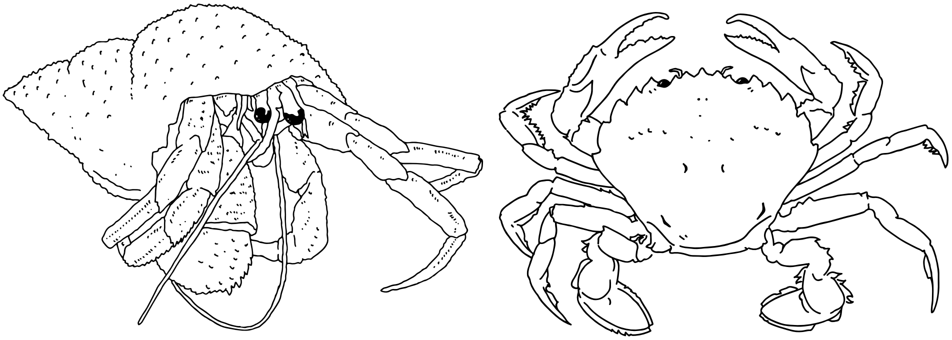
common starfish
(*Asterias rubens*)

serpent star
(*Ophiura ophiura*)



sea mouse
(*Aphrodita aculeata*)

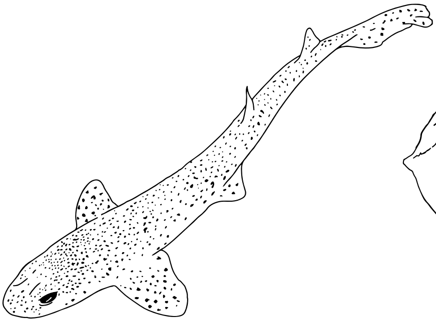
common whelk
(*Buccinum undatum*)



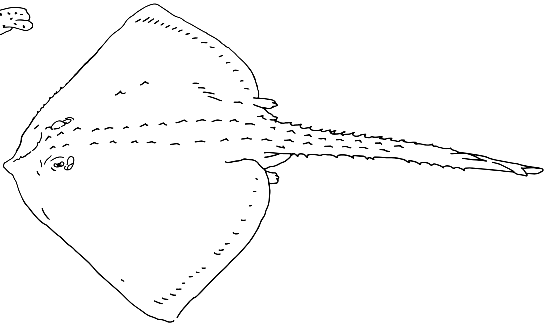
common hermit crab
(*Pagurus bernhardus*)

flying crab
(*Liocarcinus holsatus*)

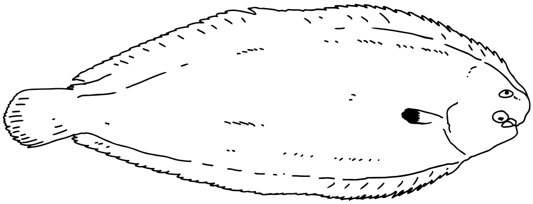
Figure 1.4. (continuation). Overview of marine animal drawings of species studied shown at the experimental research chapters (continues on next pages). Species are not drawn to scale.



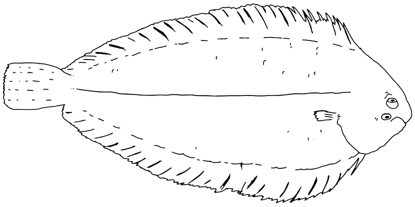
small-spotted catshark
(*Scyliorhinus canicula*)



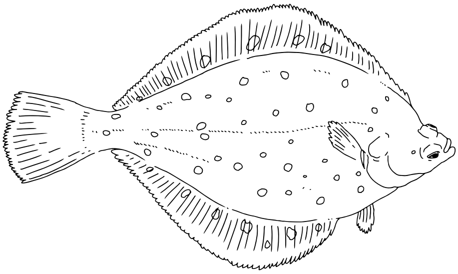
thornback ray
(*Raja clavata*)



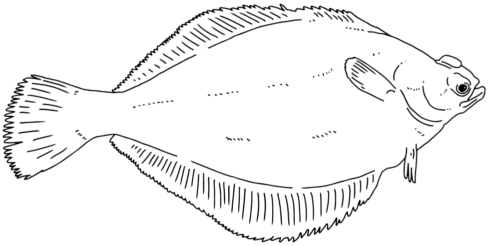
common sole
(*Solea solea*)



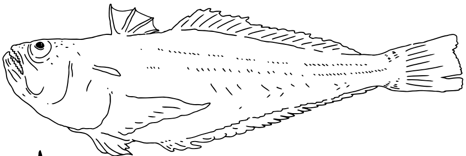
solenette
(*Buglossidium luteum*)



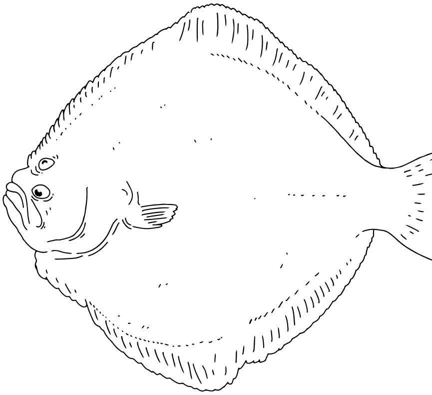
European plaice
(*Pleuronectes platessa*)



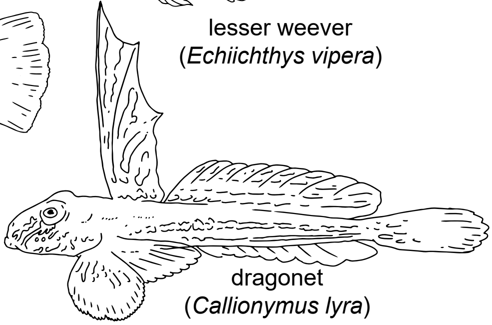
dab
(*Limanda limanda*)



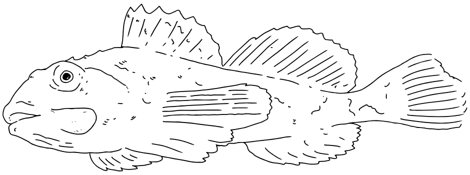
lesser weever
(*Echiichthys vipera*)



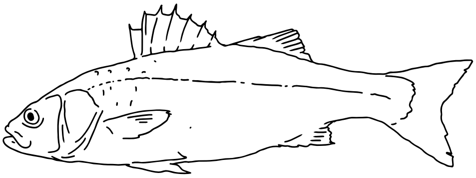
turbot
(*Scophthalmus maximus*)



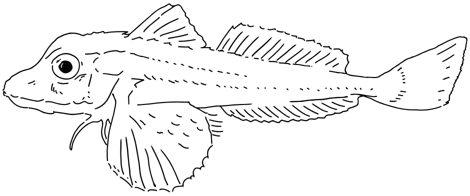
dragonet
(*Callionymus lyra*)



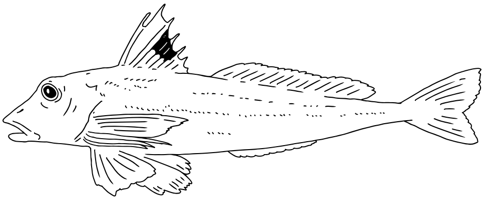
bullrout
(*Myoxocephalus scorpius*)



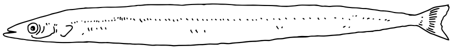
European seabass
(*Dicentrarchus labrax*)



tub gurnard
(*Chelidonichthys lucerna*)

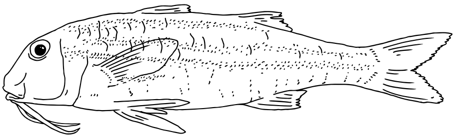


grey gurnard
(*Eutrigla gurnardus*)

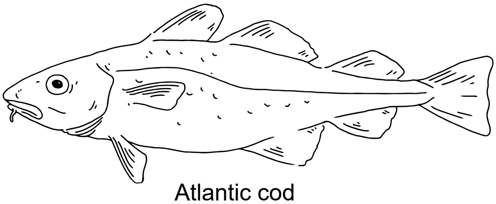


lesser sandeel
(*Ammodytes tobianus*)

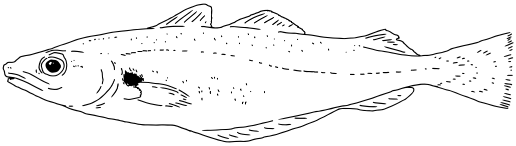
greater sandeel
(*Hyperoplus lanceolatus*)



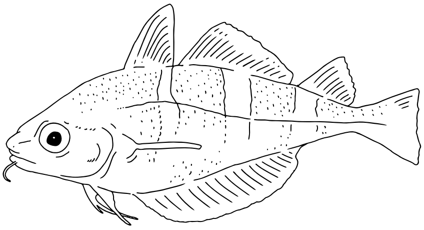
striped red mullet
(*Mullus surmuletus*)



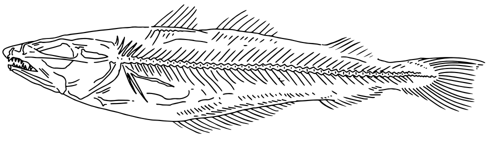
Atlantic cod
(*Gadus morhua*)



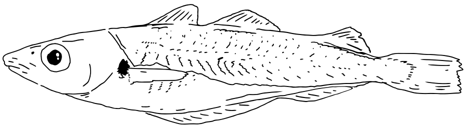
whiting
(*Merlangius merlangus*)



bib
(*Trisopterus luscus*)



whiting: X-radiograph
(*Merlangius merlangus*)



whiting: filleted
(*Merlangius merlangus*)

Finally, in the last research **Chapter 6**, we present a spinal injury assessment of sixteen fish species sampled from pulse-trawl catches with pulses turned on or off, and from tickler-chain catches by conventional trawlers, i.e. Atlantic cod, bib (*Trisopterus luscus*), bullrout (*Myoxocephalus scorpius*), common sole, dab (*Limanda limanda*), dragonet (*Callionymus lyra*), European plaice (*Pleuronectes platessa*), European seabass, greater sandeel (*Hyperoplus lanceolatus*), grey gurnard (*Eutrigla gurnardus*), lesser sandeel (*Ammodytes tobianus*), lesser weever (*Echiichthys vipera*), solenette (*Buglossidium luteum*), striped red mullet (*Mullus surmuletus*), tub gurnard (*Chelidonichthys lucerna*), and whiting (**Figure 1.4**). We used X-ray photography to quantify spinal injuries similarly as in **Chapter 5**. This interspecies comparison reveals species-specific sensitivities to injuries caused by electrical stimulation and the mechanical impact of the fishing gears. We discuss the possible mechanisms for these observations and address the ecological consequences of our findings.

In the general discussion (**Chapter 7**), we place our research findings into a wider context. Here, we integrate the thesis outcomes with the existing knowledge regarding effects of electrical stimulation on marine animals. We assess the strengths and limitations of our approach for studying electrical-pulsing effects on benthic invertebrates and fishes. We address how our results may be used to predict the effects of electrical stimulation on marine organisms in a mechanistic framework. We explore the biomimetic potential of electroreceptive and electrogenic fishes where we suggest future directions in studying electrofishing by fish for human applications. We present an outlook on future research regarding effects of electrical stimulation on marine organisms and highlight possible steps to improve catch techniques through fishing gear innovation.

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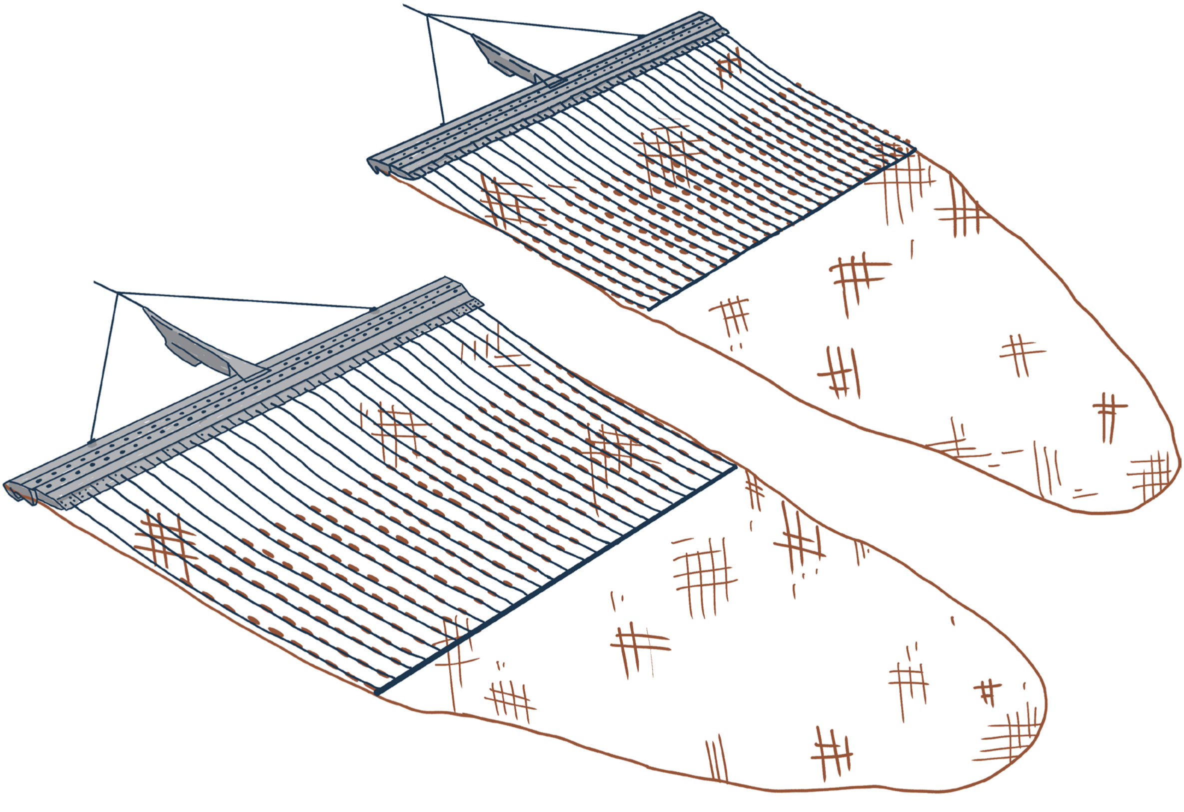

Chapter 2

Guidelines for defining the use of electricity in marine electrotrawling

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Abstract

Electricity can be used to facilitate fish and invertebrate capture in both marine and freshwater environments. In freshwaters, electrofishing is largely used for research or management purposes. In marine environments electrofishing is principally used in the form of electrotrawling for the commercial capture of fishes and benthic invertebrates, in particular common sole (*Solea solea* L.), brown shrimp (*Crangon crangon* L.), and razor clams (*Ensis* spp.). The terminology and definitions used to describe the electrical stimulus characteristics and experimental set-ups have, so far, been diverse and incomplete, hampering constructive discussion and comparison of electrofishing studies. This paper aims to (i) harmonise existing terminology, abbreviations, and symbols, (ii) offer best practice recommendations for publishing results, and (iii) provide a concise and comprehensible reference work for people unfamiliar with this topic. By incorporating common practice in marine electric pulse trawling terminology and related freshwater electrofishing studies, based on existing terms where possible, we provide a framework for future studies. The suggested guideline is recommended by the ICES Working Group on Electrical Trawling as a constructive approach to improved communication standards in electrofishing and electrical pulse stimulation research and publications.

Keywords: electrical pulse parameters, electrofishing, guidelines, ICES, pulse trawling, terminology, WGELECTRA

2.1 Introduction

The history of freshwater electrofishing goes back to the 19th century, but it was not until the second part of the 20th century that it became an important scientific fish sampling technique for population and community surveys in freshwater systems (Vibert, 1967; Snyder, 2003; Soetaert *et al.*, 2015b; Beaumont, 2016). The technique uses an electric field applied between two electrodes to induce galvanotaxis and temporary immobility, or narcosis, of the fish (Taylor *et al.*, 1957; Snyder, 2003). This allows easy and accessible collection of fish near the electrodes with a dip net (Sharber and Black, 1999; Beaumont *et al.*, 2002; Snyder, 2003).

This freshwater electrofishing knowledge was adopted in a quest to increase the catch efficiency and/or reduce fuel costs of bottom trawls by means of electrical stimulation in so-called 'electrotrawls' (e.g., Pease and Seidel, 1967; vanden Broucke, 1973; Boonstra and de Groot, 1974; Stewart, 1974; Horn, 1976, Watson Jr., 1976; Namboodirj *et al.*, 1977; Stewart, 1977; Agricola, 1985). Despite promising results in both the North Sea common sole (*Solea solea* L.) and brown shrimp (*Crangon crangon* L.) fisheries, international criticism, fuelled by fear of further increasing catch efficiency of the beam trawling fleet, resulted in a ban by the German government in 1987, the Dutch Ministry of Agriculture and Fisheries in 1988 and later in 1998 by the Council of the European Union (van Marlen, 1997; Council of the European Union, 1998; Linnane *et al.*, 2000). However, in following years around 3000 vessels in China used electrical pulses to target (mainly penaeid) shrimp (Yu *et al.*, 2007). Yet, lack of regulation and misuse of the electrical parameters resulted in a collapse of commercial shrimp stocks and a ban of this fishing method in 2001 (Yu *et al.*, 2007). After almost two decades, renewed interest led to a partial lift of the ban in the European Union by means of derogations, allowing experimental use and development of electrotrawls from 2006 onwards (Council of the European Union, 2005, 2006; Government of the Netherlands, 2014; ICES, 2018). In the following years, ~85 beam trawlers have switched to pulse trawling in the southern North Sea and reduced or replaced their conventional mechanical stimulators such as bobbins or tickler chains for electrodes generating pulsed electric fields (Haasnoot *et al.*, 2016; Sutherland *et al.*, 2016; ICES, 2018).

At present, three different types of marine electrotrawls are known to be used commercially in Europe targeting three different species: common sole, brown shrimp, and razor clams (*Ensis* spp.) (e.g., Soetaert, 2015b; Murray *et al.*, 2016). The first two types are alternatives for conventional beam trawls targeting flatfish and shrimp and are commonly called "pulse trawls" since they use pulses of electricity (i.e., a variable duration of energisation interspersed with periods of no energisation). A 1–2 s exposure of the animals to the electric field between the electrodes towed over the seabed enables fishermen to target brown shrimp and common sole (Soetaert *et al.*, 2015b). The use of this technique results in reduced fuel consumption, bottom impact, and bycatch rates (Taal and Hoefnagel, 2010; van Marlen *et al.*, 2014; Depestele *et al.*, 2016; Depestele *et al.*, 2018; Tiano *et al.*, 2019; Verschueren *et al.*, 2019). Primarily only two reactions to the electrical pulse stimulations are used to aid capture, i.e. a startle pulse for brown shrimp using a frequency (*f*) of 5 cycles per second [hertz, Hz] and a cramp pulse for common sole using around 40 Hz. However, continuous innovations by different manufacturers and changes in electrode configurations by fishermen have led to differences in pulse parameter settings used in the field (ICES, 2018). Latterly, a third type of electrotrawl exists targeting razor clams and is used in Scotland (Breen *et al.*, 2011; Woolmer *et al.*, 2011; Murray *et al.*, 2014, 2016). In contrast to the ~1 s electrical pulse stimulus used for common sole and brown shrimp, razor clams are exposed to 1 min of continuous alternating current (AC) to drive clams from their burrows where they are collected by divers or, less commonly, by dredges towed behind the electrodes. Due to the wide and increasing number of species exposed to electrical stimulation, in this document, unless a specific species is stated, the term “fish” can apply to other organisms that are being caught or affected by the electrofishing apparatus.

One of the reasons marine electrotrawling for common sole is still controversial (Stokstad, 2018), is the spinal injuries and flesh damage observed in Atlantic cod (*Gadus morhua* L.) which are bycatch in electrotrawls targeting common sole (van Marlen *et al.*, 2014; de Haan *et al.*, 2016; Soetaert *et al.*, 2016c). This drawback is also well documented in freshwater research, especially in Salmonidae, but has been reduced by optimising the waveforms pulse settings used (Snyder, 2003). Current European regulations ban the use of AC waveforms and advise on <60 Hz PDC when used in freshwater electrofishing (Anonymous, 2003). However, these settings are used by at least one marine equipment manufacturer of pulse trawls targeting common sole, which may explain why injuries in bycatch of Atlantic cod are encountered in this fishery and not in pulse trawls targeting brown shrimp using a 5 Hz square-wave PDC startle pulse (Desender *et al.* 2016; Soetaert *et al.*, 2016a). Hence marine electrotrawling targeting common sole may be optimised further by learning from electrofishing methods used to capture fish in freshwater environments. However, an ethical assessment of pulse trawling and/or optimisation of the pulse settings will be a trade-off between minimal electrically-induced harm on bycatch species such as Atlantic cod, optimal catch efficiency for the target species common sole, and other (in)direct effects on other caught species resulting from different gear riggings or fishing behaviour, e.g. by fishing at slower sailing speeds or choosing other fishing grounds. Indeed, the exposure to a single electrical stimulus of ~1 second represents only a fraction of the entire catch process (~120 minutes excl. on-deck processing), during which the captured fish are continuously being sandblasted and impacted by bycatch stones and hard-bodied invertebrates. Since pulse trawls targeting flatfish move much slower and show a large reduction in bycatch of stones and benthic invertebrates (van Marlen *et al.*, 2014), the overall impact on fish may well be smaller than conventional beam trawls. This is illustrated by undersized European plaice (*Pleuronectes platessa* L.), common sole, and dab (*Limanda limanda* L.) caught by pulse trawlers having a higher survival probability and vitality index compared to fish caught by conventional beam trawls (van der Reijden *et al.*, 2017) and by the higher price pulse trawl fishermen receive for their fish.

With the benefits that could be gained from electrotrawling it is important that structured research continues. Critical to this is a clear and thorough description of the characteristics of any electrical parameters being tested or used. Unfortunately, no consistent approach exists for the description of electrical (pulse) parameters used in marine electrotrawling laboratory and field research, creating unnecessary confusion, especially when abbreviations may have different meanings. For example, the same waveform was labelled as both ‘a 40 Hz bipolar pulse’ and ‘80 Hz pulsed bipolar current’ in studies with Atlantic cod (de Haan *et al.*, 2016; Soetaert *et al.*, 2016a, 2016b, 2016c). Furthermore, inadequate descriptions of experimental designs (e.g., tank size, and distance and orientation of the animal with respect to the electrodes) and environmental conditions (e.g., water conductivity), can make it impossible to compare studies and reveal possible causes for deviating findings. Finally, an unambiguous description is needed to properly document and monitor the settings used on vessels and to allow for control and enforcement of those regulations by local authorities.

This paper provides information on the physiological effects on organisms and physical parameters of electrical (pulse) stimulation. The paper also includes an explanation of basic principles using standard nomenclature, symbols, and units. In addition, we propose a set of definitions and abbreviations, enabling usage of harmonised terminology and descriptions of electrical (pulse) parameters in scientific publications as well as in management and enforcement documents.

2.2 Physiological responses of organisms exposed to electrical stimulation

External electrical stimulation can affect both the nervous system and muscles and is widely used in medical applications (e.g., Zoll, 1952; Basser and Roth, 2000; Peckham and Knutson, 2005). Neurons and muscle fibres use electrical signals for information transfer (e.g., Hodgkin, 1951; Hodgkin and Huxley, 1952). Neurons integrate synaptic potentials and may transmit information to other neurons or to muscle fibres via action potentials (e.g., Bullock, 1951; Fetcho, 1991). In muscle fibres, the synaptic potentials generated at the neuromuscular junction may lead to muscle contraction (e.g., Hodes, 1953; Fatt, 1954). A single action potential causes a brief and weak twitch of a muscle fibre (e.g., Hodes, 1953; Hunt and Kuffler, 1954). Larger muscle forces are produced by recruiting multiple fibres, and by increasing the frequency of action potentials, leading to temporal summation of contractive force (e.g., Hunt and Kuffler, 1954).

In fish, patterns of contraction required for swimming are coordinated by interneurons in the spinal cord, generating rhythmic and alternating contractions on the left-and-right side of the body (e.g., Uematsu, 2008; McLean and Fetcho, 2009; Fetcho and McLean, 2010). External electrical stimulation by electrofishing interferes with normal functioning by inducing action potentials in neurons and/or muscle fibres. This simultaneously stimulates both sides of the fish, leading to uncontrolled behaviour, in which mutual left–right inhibition no longer works. In freshwater electrofishing direct current (DC) or pulsed DC waveforms (PDC) are used. This leads at the positive electrode (anode) to four different responses of increasing intensity as fish are exposed to stronger electric field strengths as they get closer to the anode: fright, electrotaxis, electronarcosis, and tetanus. At the negative electrode (cathode), fright and aversion behaviours are exhibited. At increasingly intense stimulation, detrimental effects include cardiac or respiratory failure, injury, stress, and mortality; with mortality effects being both immediate or delayed. However, the specific response of an animal depends on many factors, such as species, body shape and volume, and pulse stimulation parameters, making it complex to provide a complete and conclusive overview, both for electrofishing in freshwater and marine environments. For review, see Vibert (1967a), Sternin *et al.* (1976), Beaumont *et al.* (2002), Snyder (2003), Polet (2010), and Beaumont (2016).

Marine electrotrawls generate electric fields of continuously changing polarity between two moving identical electrode arrays. As consequence, there is no electrotaxis or electronarcosis but other responses are aimed for depending on the targeted species. In electrotrawling for razor clams, the electrical settings elicit a voluntary escape response of the target species during which they emerge from the sediment; responses of non-target species vary and are species-specific (Breen *et al.*, 2011; Woolmer *et al.*, 2011; Murray *et al.*, 2014, 2016). In electrotrawling targeting shrimp, the ~1 s electrical pulse stimulus induces a startle response consisting of escape jump swimming behaviour which disperses shrimp from the sediment into the water column and makes other animals, such as fishes, twitch while still allowing them to swim voluntarily (e.g. Polet *et al*, 2005a, 2005b; Soetaert *et al.*, 2015a, 2016d; Desender *et al.*, 2016). In electric pulse trawling targeting common sole, the ~1 s electrical pulse parameters are aimed at invoking a muscle cramp response. The muscle cramp disables the fish' escape response of burrowing deeper in the sediment and makes them bend in a U-shape, after which they are scooped up by the ground rope of the fishing gear (Soetaert *et al.*, 2015b). This muscle cramp is known in both freshwater electrofishing and marine electrotrawling to potentially cause internal injuries such as fractures and dislocations of the vertebral column, which may be accompanied by haemorrhages (Snyder, 2003; van Marlen *et al.*, 2014; de Haan *et al.*, 2016; Soetaert *et al.*, 2016a, 2016b, 2016c). These side effects result from simultaneous electrically-induced muscle contractions at both sides of the fish' body, an unnatural response because mutual inhibition via interneurons in the spinal cord normally prevents simultaneous contractions of left-and-right swimming muscles in fish (e.g., Uematsu, 2008; Fetcho and McLean, 2010) and mainly occurs in fusiform fish with a high number of small vertebrae such as trout and salmon species (Snyder, 2003) or Atlantic cod (Soetaert *et al.*, 2018).

2.3 Electric principles of electrofishing

An electric field is generated in the water by a power supply that provides power to electrodes in which the charge flows between the negatively charged electrode(s), i.e. cathode(s), and the positively charged electrode(s), i.e. anode(s) (Snyder, 2003; Beaumont, 2016). In the context of electrofishing, 'electrodes' are the conductive parts of the electric circuit in contact with the water. The electrodes may be mounted on, or separated by, non-conducting elements (insulators) which together can be termed the electrode array (**Figure 2.1**). These descriptions will be applied throughout the manuscript and are strongly advised to be adopted in future research.

When a circuit with electrodes placed in water is charged, a potential difference (V, [volt, V]) is generated between the electrodes. Charged ions will flow between the anode and cathode and induce an electrical current (I, [ampere, A]) in the water between the electrodes. The amount of current between the electrodes at a given potential difference is related to the electrical resistance (R, [ohm, Ω]) of the circuit according to Ohm's law, ($V = I \times R$). Electrical resistance measures the difficulty an electric force encounters when passing a current through a circuit. Resistivity measures how strongly a given substance opposes an electric current (ρ, [ohm-metre, Ω·m]). When measuring the ability of a unit of volume of water to conduct electricity it is usual for the reciprocal value of resistance ($1/R$) to be used, this is termed its conductivity (σ, [siemens per metre,

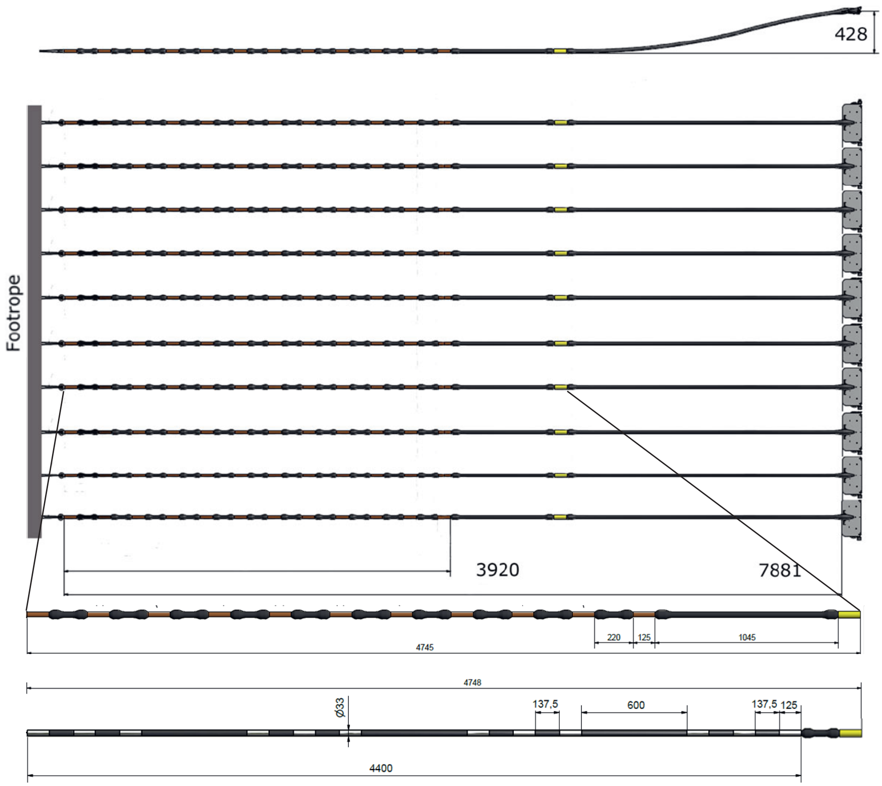

Figure 2.1. Schematic representation (dimensions in mm) of the ten 7.881 m long electrode arrays of a 4 m beam pulse wing used in electrotrawls targeting common sole with a close-up of two possible electrode-array types (from HFK Engineering B.V.). The white or grey conductive parts are made of stainless steel or copper respectively and are called electrodes, whereas the longer black parts are non-conductive and called insulators or insulated parts. The entire structure consisting of electrodes and insulators through which the pulse generator releases its electrical current is called an 'electrode array'. Note that 'electrode array', 'electrode' and 'insulator' were often referred to as 'electrode', 'conductor' and 'isolator' respectively in older electrotrawling manuscripts. It is strongly advised to no longer use the older terminology in future research.

S m^{-1}] or [microsiemens per centimetre, μS cm^{-1}]). This conductivity depends on the amount of total dissolved ions in the water (e.g., calcium, sodium) and temperature of the water (UNESCO IES 80). As temperature affects conductivity (and resistivity) the value of conductivity is usually normalised to what it would be at 25 degrees Celsius (specific conductivity) rather than the conductivity at the ambient temperature of the water (ambient conductivity). When describing conductivity it is important to specify which metric is being used and the water temperature. For electrode arrays in water, their resistance is comprised of several component factors: the resistance of the metal elements of the electrode in air (normally minimal), the geometry of the electrode, the distance apart of the electrodes, and the resistivity/conductivity of the water. This combined electrode array resistance is termed the equivalent resistance of the electrode (R_{eq}). Together with applied voltage it is this metric that determines the power demand of the fishing equipment (Beaumont *et al.*, 2005).

The energy transfer rate of a generator is the power (P, [watt, W]) and can be calculated in three ways: $P = V * I$, $P = I^2 * R$, or $P = V^2 / R$. However, where AC generators are used for certain electrical equipment (e.g., motors and transformers), time lags between voltage and current (phase shift) in the components leads to more power being needed than the theoretical, or apparent power ($|S|$, [volt-ampere, VA]). This disparity is resolved by using a power factor correction (PF) to multiply the apparent power: i.e. $P = |S| * PF$. Power factor (from a source to a load) can vary, depending on the equipment, between 1 and 0, with 1 being no power loss. For example, equipment with a 0.5 PF would draw 50% more power than one with a PF of 1 and therefore, if the apparent power demand was 1000 VA, it would need 1500 W to run the equipment. This leads to larger power sources being needed. The increase in generator capacity needed due to PF is one reason why the use of AC waveforms is attractive to operators. However, the use of capacitors within the power distribution circuit can reduce the power factor. For bankside electrofishing equipment used in freshwater environments a PF of 0.6 is commonly used.

The voltage difference between a pair of conductors generates an electric field which is characterised by its strength and orientation. The electric field defines the current flow at each location and can be visualized by electric field lines, indicating the direction of current flow at each location. Alternatively, one can define equipotential lines that run perpendicular to the electric field lines and indicate directions in which there is no net current flow (**Figure 2.2**). The potential difference between two sequential equipotential lines is an arbitrary but constant value. Consequently, the distance between subsequent equipotential lines indicates the electric field strength or voltage gradient (E, [volt per metre, V m^{-1}] or [volt per centimetre, V cm^{-1}]). The electric field can also be described by the two-dimensional current density (J) which is the electric current per cross-sectional area of its path [ampere per square centimetre, A cm^{-2}] (Sternin *et al.*, 1976). Current density can be calculated by multiplying the voltage gradient E with the water conductivity (σ). An additional method of describing the amount of power that needs to be transferred into a fish to achieve, for example, immobilisation and tetanus, called power density (D, [watt per cubic centimetre, W cm^{-3}]), was proposed by Kolz and Reynolds (1990). Power density is calculated from J^2/σ. As transferred power density values for e.g. immobilisation are constant across water conductivities they allow standardisation of outputs for different water conductivities (Kolz and Reynolds, 1990; Burkhardt and Gutreuter, 1995; Snyder, 2003; Beaumont, 2016). Although voltage gradient is easier to measure, it is the current and/or power density that is the most significant factor in determining fish's reaction to an electric field.

If two large and flat, plate-shaped conductors are used, electric field lines will be equally distributed in the water volume and run parallel (i.e., create a homogeneously-distributed electric field), whilst equipotential lines are oriented in parallel with the conductors' surface (**Figure 2.2A**). This set-up's advantage is its predictability: the electric field

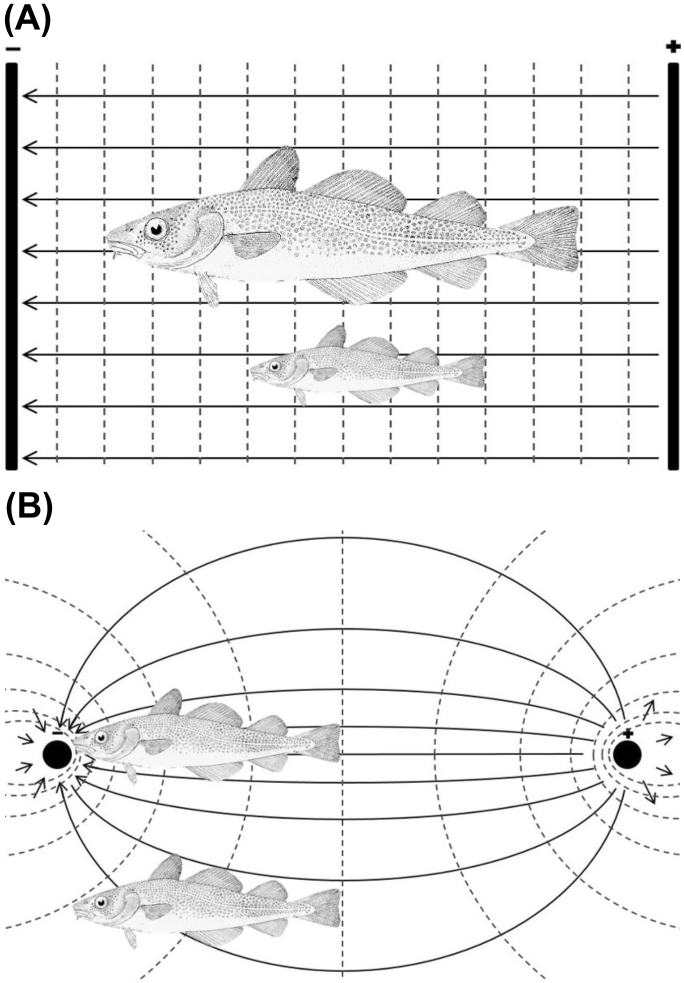

Figure 2.2. Schematic representation of fish in **(A)** a homogeneous and **(B)** heterogeneous electric field (Soetaert *et al.,* 2015b). The fish in these hypothetical scenarios have the same conductivity as the surrounding medium and therefore do not affect the electric field. The solid black structures represent an electrode pair between which an electric field is formed (heavy black lines and dots in the top and bottom panel respectively). The arrows indicate the electric field vectors representing the current flow. The dashed lines are equipotentials representing regions with the same potential. If more equipotential lines cover the fish's body, a larger potential difference, hence a higher current density, is present over its body.

strength is constant and uniform and is easily calculated by dividing the applied voltage by the distance between the two conductors. Moreover, the extremities of an animal placed in a homogeneous field will have a constant potential difference, regardless of their position, as long as their orientation remains unchanged. Hence, homogeneous electric fields are used in laboratory set-ups to study the effects of electrical stimulation on organisms, since this design enables standardisation with minimal variability in field strengths (Soetaert *et al.*, 2015a, 2016a). Note that in a natural environment many factors can distort the idealised model of the electric field propagated from electrodes, e.g., by conductive objects being within the field.

In freshwater electrofishing the anodes are usually sphere, ring (torus), or rod-shaped electrodes. Cathodes are usually high surface area grids or braided ribbon, which create a low electrical resistance electrode, and thus low field density. In marine electrotrawling, the anode and cathode are always rod shaped and of the same size within an electrode array and fishing gear (**Figure 2.1**). This results in a heterogeneously-distributed electric field (**Figure 2.2B**). Near to the direct surroundings of the electrodes voltage gradient is high, indicating high current density, which decreases with distance from the electrode (Beaumont *et al.*, 2006; de Haan *et al.*, 2016). Hence, the electrode position relative to the fish, can result in a relatively large increase or decrease of the electric field strength experienced (Soetaert *et al.*, 2015b; Beaumont, 2016). Therefore, free-swimming fish will experience a wide range of reactions to an electric field depending on their distance to and orientation in the field.

2.4 Variables affecting the electric field distribution

Various environmental variables may affect the shape and intensity of the electric field and consequently the effect on exposed animals. Below, we outline the major components that may constitute these effects on the electric field, i.e. the water, sediment, and electrode array characteristics.

2.4.1 Water

The equivalent resistance of the electrodes determines which electrical settings can be achieved within the limitations of the electrofishing generators being used. As power can be calculated by dividing the voltage squared by resistance (see earlier), higher conductivity water (lower resistivity) will require more power since the equivalent resistance is lower ($P = V^2/R_{eq}$). The conductivity of fish in relation to the surrounding water is important because it determines the amount of electric current transferred from the water to the fish (Whitney and Pierce, 1957; Snyder, 2003). Kolz (1989) also considered that the mismatch between the fish and water conductivity affected the power transferred into the fish and thus the fish's reaction to the electric field. The relationship between the conductivity of the fish and the surrounding medium leads to a concentrating or dissipating effect of the electric field (**Figure 2.3**; Sternin *et al.*, 1976) and fish in higher conductivity water will experience a higher current density compared with lower conductivity water (Sternin *et al.*, 1976, Snyder, 2003). This conductivity mismatch results in lower voltage gradients being required to generate sufficient power density to incapacitate the fish in high conductivity water compared to low conductivity water. For example, at very low conductivity water (<20 µS cm^{-1}) voltages of >1000 V are needed to induce narcosis (Beaumont, 2016) compared to 45–65 V used in marine electrotrawling (Soetaert *et al.*, 2015b). The presence of other fish nearby also affects the electric field experienced by an individual as, in case of seawater, the electric field will be 'concentrated' in a smaller volume of water, hence increasing the electric field strength experienced by an individual fish, as illustrated by D'Agaro and Stravisi (2009). In addition, the variable conductivity of different fish species (Halsband, 1967) may affect reactions, although for simplicity Burkhardt and Gutreuter (1995) used a fixed value for effective fish conductivity of 150 µS cm^{-1} with PDC waveforms (Kolz and Reynolds, 1990).

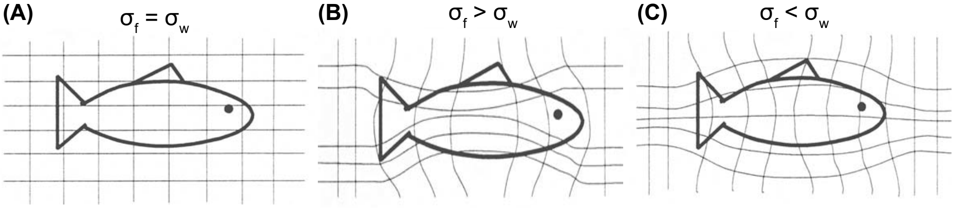

Figure 2.3. Schematic representation of three idealised distortion patterns of an electric field surrounding a fish with varying relative values of the electrical conductivity of the fish (σ_f) and the ambient conductivity of the water (σ_w) (from Kolz, 2006). The horizontal and vertical lines represent the current lines and the equipotentials, respectively. In **(A)**, the conductivity of the fish is the same of the surrounding water (i.e., as used in **Figure 2.2**). In **(B)**, the fish has a higher conductivity compared to the surrounding water (i.e., relatively low conductivity freshwater), which results in lower voltage gradients in the fish compared to the surrounding medium, whereas the voltage gradients in the fish in **(C)** (i.e., relatively high conductivity seawater) will be higher compared to the surrounding medium.

2.4.2 Sediment

Composition and structure of the sediment may also affect the shape and intensity of the electric field. Factors impacting the electric field distribution in the sediment are particle grain size (i.e. porosity), determining the amount of water present in the sediment, and the amount of organic matter between the inorganic particles (Zalewski and Cowx, 1990). Measurements by de Haan and Burggraaf (2018) indicate that electric field strengths are almost evenly distributed in the water volume and the sediment when electrodes are placed on the sediment, although field strengths measured in the sediment were slightly higher than those in the water column at equal distance. Field strengths measurements in the sediment, as well as the variability between replicates, tended to be higher in muddy sediment when compared to the more compact sandy sediment. Consequently, depth of the substrate layer in laboratory experiments, as well as the dimensions and building material of the exposure tank, will affect the electric field distribution around the electrodes. Interactions between the electric field and the sediment, or water surface, are termed boundary effects.

2.4.3 Electrode (array) characteristics

The equivalent resistance of the electrodes is a function of size, shape, surface area, and spacing. High surface area electrodes will have a low resistance and will have a lower probability of injuring fish, because the maximum electric field near the electrodes will be lower compared to electrodes with a smaller surface area (when using the same potential difference and distance). Hence, large electrodes are preferred to minimise injuries (Snyder, 2003; Beaumont *et al.*, 2006).

In marine electrofishing, high water conductivity leads to lower voltage levels being needed to achieve an effective electric field density. Electrode arrays used in marine electrotrawling are either long thin electrodes (1.5 m x ⌀12 mm) or multiple short electrodes (160–180 mm x ⌀~40 mm) alternated by insulators on the electrode arrays, which are towed over the seafloor (**Figure 2.1**). By having electrodes of this design, the equivalent resistance of the electrode(s) is increased and thus the power demand reduced. Due to the high power demand of the electrode array in sea water, the pulse shape may be affected if the power supply is not sufficient, e.g., square waveforms having a falling voltage after an initial peak value. It is important to note that when operating multiple electrode arrays using pulsed waveforms in close proximity, pulses are likely to be out of phase and thus create high (potentially damaging) frequencies in the area where the electric fields overlap (Beaumont, 2017).

2.4.3 Movement

Movement of the fish and/or electrode arrays affects the time duration the fish is exposed to the electrical pulse stimulus. A fish swimming over a stationary, wire-shaped electrode pair will be exposed to varying electric field strengths, experiencing maximum intensity when located closest to the electrodes. If a moving electrode array is used, as in commercial fishing practice, the exposure will also depend on the location of the animal relative to the electrodes plus its ability to move during exposure. For example, pulse trawling using an immobilising stimulus such as the cramp pulse for common sole, allows for calculation of the maximal total exposure time by dividing the length between the start of the first and the end of the last electrode element by the towing speed of the gear relative to the bottom. However, this may be much shorter if the animal is exposed in the periphery of the electric field or exposed to a startle pulse and able to escape the electric field. Besides, the exact exposure intensity depends on the location and orientation of the organism with respect the electrodes. An electrode array consisting of multiple electrodes, moving faster than the organism is able to escape, will expose the animal to a complex pulse train consisting of different short exposures, each of them rising and waning in strength (de Haan *et al.*, 2016).

2.5 Electrical waveform parameters

Two main types of electric current exist: DC and AC. However, to cope with the high energy demand in high conductivity environments such as seawater, a series of short electrical pulses instead of continuous current flow are used for electrical pulse trawling. In marine electrofishing, pulses are often produced by using a capacitor to accumulate and then quickly discharge electric current. Hence, the same peak power that is delivered in a continuous DC waveform is now released during a pulse with a shorter duration, thus reducing mean power demand. The resulting waveform is a PDC but PBC and pulsed alternating current (PAC) can be delivered when H-bridges are used to switch connection between the two electrode arrays.

2.5.1 Terminology used for describing electrical waveform parameters

Pulsed electrical waveforms are characterised by recurring patterns of individual pulses of current. The complete sequence intervening between two successive corresponding points in that pattern is termed the cycle of the waveform. In pulsed currents, the distinction should be made between the “cycle”, (see definition above) and an individual “pulse”, i.e. a single pulse of electric current, which may encompass a complete cycle (in PDC waveforms) or be a part of it (in AC, PBC, and PAC waveforms). PDC, PAC, and PBC waveforms can be described by electrical pulse parameters illustrated in **Figure 2.4** and defined in **Table 2.1**.

Table 2.1: Overview of electrical pulse parameters with their symbol, unit, and definition.

Category	**Pulse parameter or waveform characteristic**	**Symbol**	**Unit or 'expressed in'**	**Definition**
Key parameters	Amplitude	V	Volt, V	Maximum potential difference or field strength of a pulse. This can be circuit or location specific and be expressed as peak voltage, peak-to-peak voltage, median voltage, or root mean square voltage.
	Frequency	f	Hertz, Hz	Number of cycles per second.
	Pulse width	PW	millisecond, ms	Time duration that the pulse is on.
	Pulse shape	PS	-	Shape of a single pulse which can be, e.g., exponential decay, sinusoidal, or rectangular (see Snyder, 2003).
Amplitude	Peak voltage	V_{pk}	Volt, V	Magnitude of the zero to maximum (or minimum) instantaneous voltage appearing between the electrodes. If a poorly formed waveform is used with an initial voltage overshoot (**Figure 2.4**) then V_{pk} will reflect this value. If using bipolar pulses, which have positive and negative peaks with different amplitudes, the highest absolute value should be given.
	Peak-to-peak voltage	$V_{pk\text{-}pk}$	Volt, V	Potential difference between the maximum and minimum instantaneous voltage appearing between the electrodes. For PDC (with no negative component), $V_{pk\text{-}pk}$ will equal V_{pk} since all peaks have the same polarity and are measured against the baseline. For alternating/bipolar pulses, $V_{pk\text{-}pk}$ is the potential difference between the positive and negative peak voltage: $V_{pk\text{-}pk} – V^{+}_{pk} – V^{-}_{pk}$.
	Median voltage	V_{med}	Volt, V	Voltage measured in the middle of a pulse, i.e. at half the *PW*. Although this value does not properly represent the energy content, it is easy and straight forward to interpret and determine for rectangular pulse shapes. It also diminishes the impact of voltage overshoot at the onset or end of the pulse and gives a measure of pulse stability or decay.
	Root mean square voltage	V_{rms}	Volt, V	Equal to the value of DC voltage that would produce the same power dissipation in a resistive load.
Time-related parameters	Duty cycle	dc	Percentage, %	Calculated as $dc = \left(\frac{PW \times f}{1000}\right) \times 100$ for PDC or $dc = \left(\frac{(PW_1 + PW_2) \times f}{1000}\right) \times 100$ for PAC and PBC with the pulse width (*PW*) in milliseconds and frequency (*f*) in Hz.
	(Inter pulse) interval time or pulse break time	PB	Millisecond, ms	Time span between two pulses, measured from the end of the fall time to the onset of the rise time of the next pulse.
	Period	T	Millisecond, ms	Time from the start of one cycle to the start of the next cycle, i.e. 1000/*f*.
	Pulse period	PT	Millisecond, ms	Time from the start of one pulse to the start of the next pulse, i.e. $PW + PB$. Note that for PDC, $PT = T$.
	Rise time	δt_{rise}	Millisecond, ms	Time it takes the pulse to rise from 10% to 90% of V_{med}.
	Fall time	δt_{fall}	Millisecond, ms	Time it takes the pulse to fall from 90% to 10% of V_{med}.
Other parameters	Total pulse width	PW_t	Millisecond, ms	Time interval in PAC covering both pulses $PW_t = PW_1 + PB_1 + PW_2 = T – PB_2$.
	Apparent frequency	f_a	Hertz, Hz	Number of PBC pulses per second.
	Burst width	BW	Millisecond, ms	Time duration that a PB pulse is present starting from the onset of the first pulse until the end of the last pulse of the burst.
	Burst interval/ break time	BB	Millisecond, ms	Time interval between two bursts of a GB.

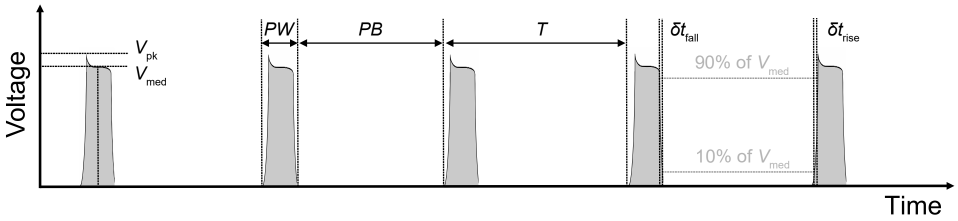

Figure 2.4: Schematic representation of square-wave PDC with overshoot. The indicated waveform parameters are peak voltage (V_{pk}), median voltage (V_{med}), pulse width (*PW*), pulse interval or break time (*PB*), period (*T*), fall time (δt_{fall}) and rise time (δt_{rise}). If the presented time frame is considered on scale with a total duration of 1 s, the frequency would be five cycles per second (*f* = 5 Hz), the pulse width 40 ms and the duty cycle (*dc*) 20%.

In previous pulse trawling research, PAC has been used to refer to the waveform type where the polarisation reversal occurred (almost) immediately followed by a long (inter pulse) interval time, whereas PBC was used when the interval time between the polarisation reversal was equal (**Figure 2.5**) (Soetaert *et al.*, 2016a, 2016b). We propose to make the distinction threshold between PAC and PBC based on the length of the pulse width (*PW*) and pulse break time (*PB*). All bi-directional waveform types of which the shortest *PB* exceeds the longest *PW*, should be referred to as PBC, and otherwise as PAC. This approach clarifies the difference between both waveform types, but it does not overcome inherent confusion about the pulse width and break time variations. Therefore, we recommend to include pulse width and pulse interval time/break time in the name of the applied waveform type, especially when different waveform types are used and discussed in the same study. This should be done by firstly indicating the pulse width, followed by the break time between brackets. The pulse followed by the shortest *PB* is considered the first with its *PW* and following *PB* referred to as PW_1 and PB_1, whereas the next pulse *PW* and *PB* are referred to as PW_2 and PB_2 (**Figure 2.5C**). In case of PAC, 40 Hz PAC (*PW* = 0.2 and 0.3 ms, *PB* = 0.1 and 24.4 ms) is a bi-directional waveform of which each period consists of a 0.2 ms pulse, a 0.1 ms interval, a 0.3 ms pulse from the opposite polarity and a 24.4 ms interval (**Figure 2.5C**). In case of PBC, 40 Hz PBC (*PW* = 0.3 and 0.2 ms, *PB* = 12.25 and 12.25 ms) is a bi-directional waveform of which each period consists of a 0.3 ms pulse, a 12.25 ms interval, a 0.2 ms pulse of opposite polarity, and another 12.25 ms interval, as illustrated in **Figure 2.5D**. In case both pulse widths and/or both interval times have the same duration, it suffices to give the value once. For example, PBC (*PW* = 0.25 and 0.25 ms, *PB* = 12.25 and 12.25 ms) can be rewritten as PBC (*PW* = 0.25 ms, *PB* = 12.25 ms) and PAC (*PW* = 0.25 and 0.25 ms, *PB* = 0 and 24.5 ms) as PAC (*PW* = 0.25 ms, *PB* = 0 and 24.5 ms) (**Figure 2.5E**). Although only indispensable for a concise but clear notation of PAC and PBC, this can also be applied to PDC. For example, pulse type 80 Hz PDC (*PW* = 0.25 ms, *PB* = 12.25 ms) (**Figure 2.5A**). In addition, it is also proposed to introduce the total

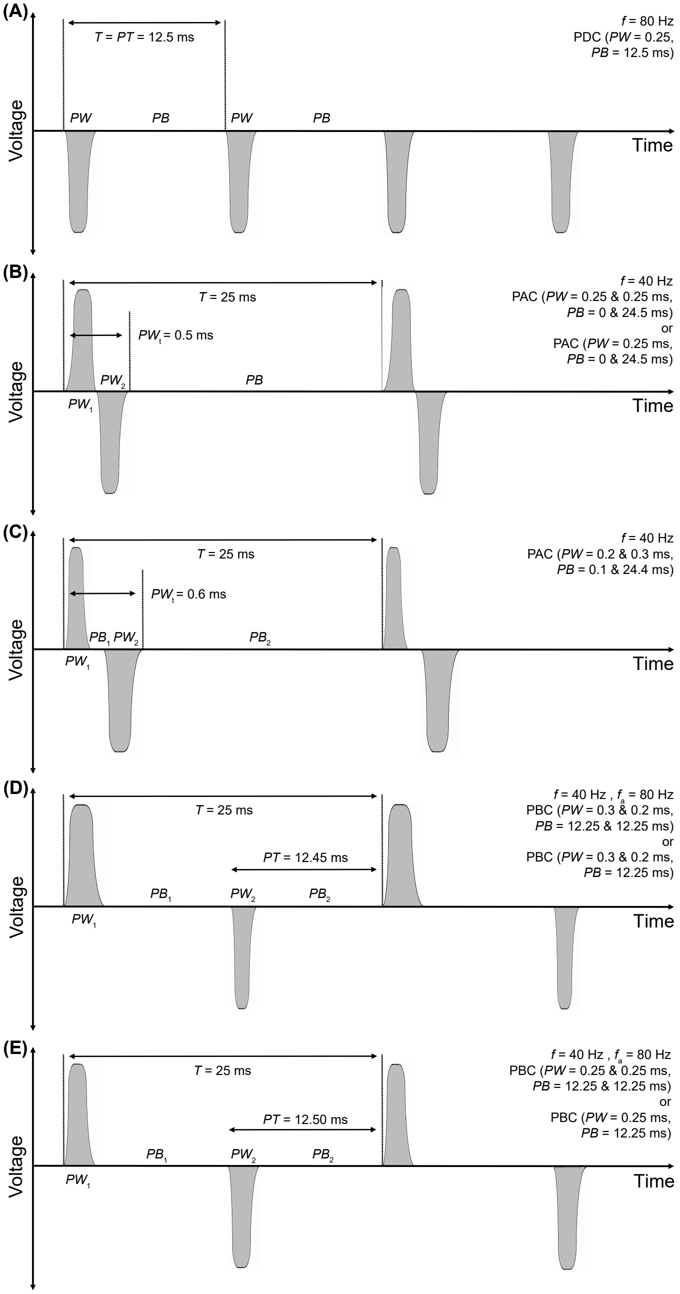

Figure 2.5. Schematic representation of **(A)** PDC; **(B)** and **(C)** PAC; and **(D)** and **(E)** PBC waveforms to illustrate the pulse parameters and pulse names. Each depicted pulse stimulus (not on scale) has a duty cycle of 2%. The indicated pulse parameters are pulse width (*PW*), total pulse width (PW_t), pulse break time (*PB*), period (*T*), pulse period (*PT*), pulse frequency (*f*), i.e. the number of cycles per second, and the apparent frequency (f_a), i.e. the number of PBC pulses per second. The legend right above each x-axis indicates the frequency as well as the recommended name to describe that specific waveform.

pulse width (PW_t) as the time interval in PAC covering both pulses: $PW_t = PW_1 + PB_1 + PW_2 = T - PB_2$ (**Figure 2.5B**, **C**).

2.5.2 Gated bursts

Pulsed electrical waveforms can also be provided as gated bursts (GBs). These are complex pulse stimulations consisting of short series of higher-frequency pulses (referred to as bursts) delivered at a lower secondary frequency as illustrated in **Figure 2.6**. This pulse stimulation type is claimed to reduce the incidence of spinal injuries in freshwater electrofishing by inserting periods with reduced pulse stimulation allowing for the relaxation of the muscles (Snyder, 2003). It also considerably reduces the mean power demand (i.e. V_{rms}, A_{rms}) of the output. We suggest application of a similar approach as shown above to describe GB by using the concept of burst width (*BW*, [milliseconds, ms]), expressed as the time duration that the pulse is present starting from the onset of the first pulse until the end of the last pulse of the burst, and burst interval/break time (*BB*, [milliseconds, ms]), i.e. the interval time between two bursts (**Figure 2.6**). For example, a pulse train of 5 Hz, with each series of pulses containing 5 DC pulses at a frequency of 100 Hz (PDC ($PW = 0.2$ ms, $PB = 9.8$ ms)) followed by a 159.8 ms break would be named GB ($PW = 0.2$ ms, $PB = 9.8$ ms and $BW = 40.2$ ms, $BB = 159.8$ ms) (**Figure 2.6**).

2.5.3 Physiological relevance of unambiguous waveform parameter definitions

Confusion can arise when comparing PAC and PBC results since the frequency can be interpreted differently. Indeed, the physiological effect of the 20 Hz PBC is similar to that of the 40 Hz PDC, assuming the same voltage and duty cycle, because the neuromuscular system will experience 20 negative pulses plus 20 positive pulses (i.e. 40 individual pulses) per second. When aiming to induce muscle cramp, the temporal summation of electrical stimuli determines the contractive force. Some studies focusing on physiological effects therefore listed the PBC frequency as the number of individual pulses as this was most relevant to compare responses between PDC, PAC, and PBC (Soetaert *et al.*, 2016a, 2016b) because a 20 Hz PBC ($PW = 0.25$ ms, $PB = 12.25$ ms) with 40 pulses per second would induce tetany whereas a 20 Hz PDC ($PW = 0.5$ ms, $PB = 12$ ms) or 20 Hz PAC ($PW = 0.25$ and 0.25 ms, $PB = 0$ and 24.5 ms) would not. However, this frequency was incorrect and should have been divided by two, since frequency is expressed as the number of unique cycles per second, i.e. each repetition of a positive and a negative pulse. Hence, we suggest to differentiate between frequency (*f*), i.e. the number of cycles per second, as defined by the International System of Units (**Figure**

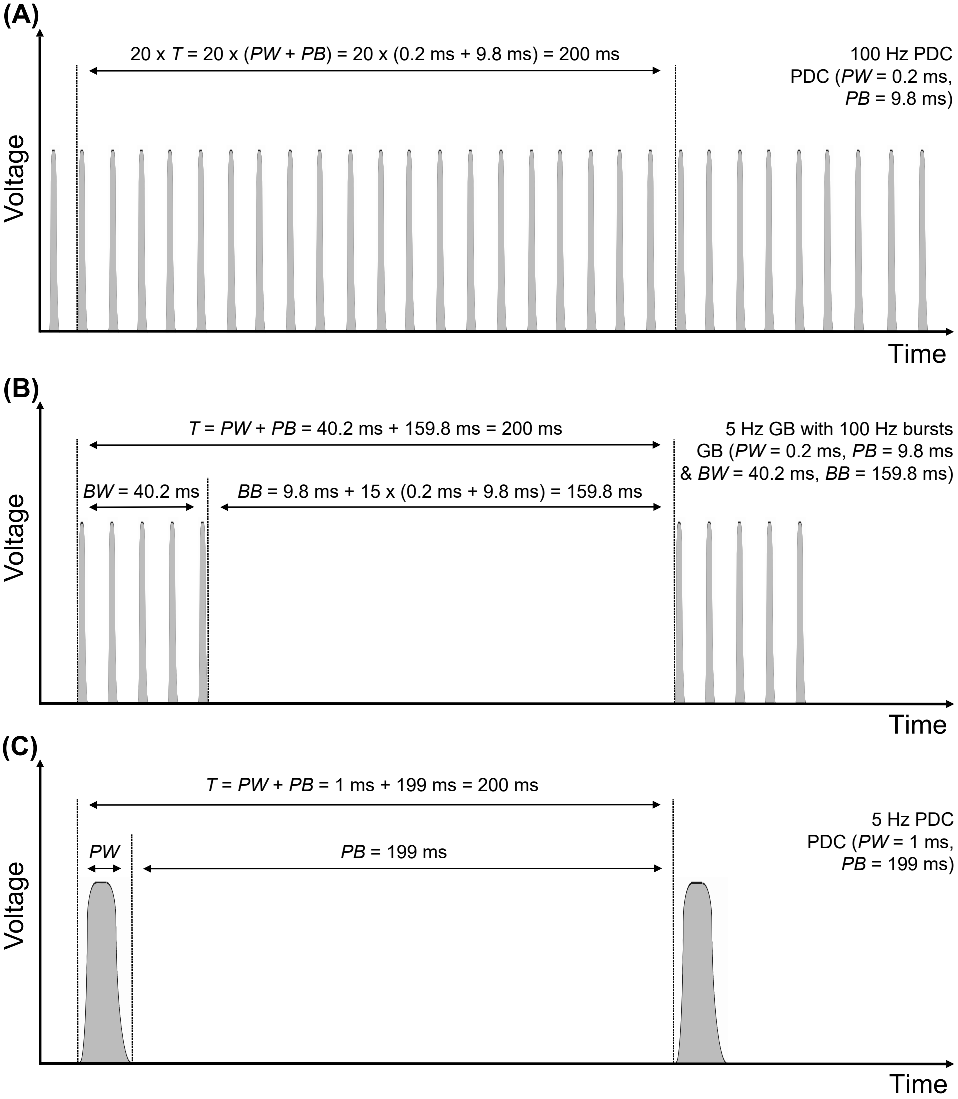

Figure 2.6: Schematic representation of a 100 Hz PDC **(A)** used to generate the GBs **(B)** and a 5 Hz PDC with the same period (*T*) as the GB **(C)**. The indicated waveform parameters are pulse width (*PW*), burst width (*BW*), inter pulse interval or break time (*PB*), and burst break time (*BB*). The legend in the top right corner of each graph indicates the frequency (*f*) as well as the recommended name to describe the specific waveform. The 100 Hz PDC has a duty cycle of 2% whereas the GB and 5 Hz PDC both have a duty cycle of 0.5 %.

2.5D, **E**), and the “apparent frequency” (f_a), i.e. the number of individual PBC pulses per second. The apparent frequency of a PBC frequency of 20 Hz would therefore be 40 Hz (**Figure 2.5**). If not specified, “frequency” should always refer to the number of cycles per second.

2.6 Standardising study design descriptions in laboratory, computational, and field set-ups

The intensity of the electric field at a certain location depends on many factors such as the electrode characteristics, tank configuration, stream characteristics, position of the animal, animal body plan and characteristics, and the specific waveform parameters used, as seen in previous chapters. Hence, clear and complete descriptions of the field or experimental set-up designs are required for qualitative and quantitative repetition of results. **Table 2.2** gives a guideline to do so in a standardised way indicating what information should be provided recommended or optionally. The minimum elements needed to recreate the experiment are given in the column 'Recommended' whereas other items of interest are given in column 'Optional'.

Although the use of an oscilloscope image is not strictly necessary, it is highly recommended to include when presenting data because it helps to visualise and check the waveform parameters used. Ideally this should consist of two parts: an overview of the waveform on a time frame of ~1 s (**Figure 2.7A**) and a close-up of a single pulse (**Figure 2.7B**) on which the time and voltage intervals are given. In case of a gated burst, a third figure showing one entire burst cycle is recommended. Additionally, other relevant waveform and pulse parameters, as well as their values measured by the oscilloscope, can be indicated in the image or caption.

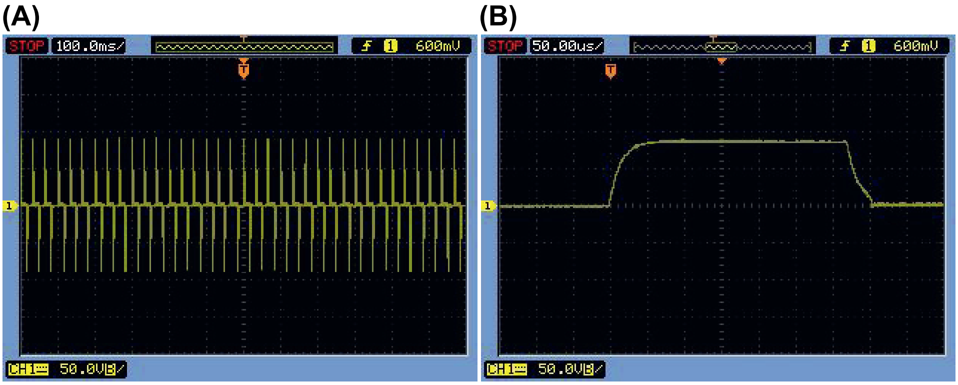

Figure 2.7. An overview of the same square-wave or rectangular electrical pulse stimulus plotted at a time frame of 1.2 s (12 x 100.0 ms) **(A)** and 0.6 ms time frame showing the single pulse (12 x 50.0 µs) **(B)**. This pulse stimulus was generated in seawater using the following settings: frequency (*f*) = 30 Hz, amplitude = 85 V, and pulse width (*PW*) = 0.33 ms. The graphs show the measurements taken on the electrodes in the water indicating 3 cycles per interval of 100.0 ms or 30 cycles per second (Hz) and a rise time (δt_{rise}) and fall time (δt_{fall}) of 0.05 ms and 0.03 ms, respectively. The pulse width is 0.35 ms instead of the set 0.33 ms which is due to the extended fall time sometimes caused by certain electrical circuits or pulse generators, which illustrates the importance of verifying the output pulse parameters by means of an oscilloscope.

Table 2.2: Overview of information to be provided when describing an electrofishing set-up for experimentation. The minimum elements needed to recreate the experiment are given in the column "Recommended" whereas other items of interest are given in column "Optional".

Information category	Recommended	Optional
Generator equipment	• Manufacturer • Model number • Rated power output	° Supply type (mains and generator) ° Supply output (volt and ampere) ° Presence of e.g. capacitors, inductors, and H-bridges
Electrode array(s)	• Dimensions and number of electrode (array)s and insulators • Construction material • Positioning in tank or fishing gear • Distance apart (height or linear distance)	° Description of equipment used to position the electrodes in the tank ° Figure of the electrode set-up
Water characteristics	• Water depth • Conductivity (ambient or specific) • Temperature	° Salinity ° Dissolved oxygen ° pH ° Ammonia, nitrite, nitrate
Experimental tank	• Dimensions • Construction material • Porosity/particle size of bottom substrate • Depth of bottom substrate • Construction of any fish holding device/net • Schematic drawing and/or photo of set-up	° Presence of other (conducting) objects/materials in the tank such as filtration tubing and pumps ° Field characteristics (homogeneous or heterogeneous)
Experimental animal	• Species • Acclimatisation period in tank • Orientation of the animal relative to the electrodes • Animal size and mass • Anaesthetics: use, type, and dose	° Origin of animal (wild/reared) ° Animal sex ° Reproductive stage (e.g. immature, mature, or gravid) ° Any feeding regime ° Number of animals exposed simultaneously ° Presence and location of wounds/ lesions/malformations (prior and/ or after experiment/electrical exposure) ° Number of vertebrae
Waveform parameters	• Waveform type: PDC, PBC, PAC, or GB • Pulse shape • Pulse frequency • Pulse and/or burst width • Pulse amplitude (e.g. V_{pk}, $V_{pk\text{-}pk}$, V_{rms}) • Pulse exposure duration	° Duty cycle ° Pulse or burst break/interval time ° Pulse rise time ° Pulse fall time ° Oscilloscope image of waveform

Finally, we also suggest standardising the usage of measurement units but these are not restrictive and may be adjusted, depending on the area of interest, to achieve the appropriate descriptions. For example, expressing voltage gradient in V m^{-1} is common practice in marine electrotrawling, due to lower voltage gradients used, in contrast to freshwater electrofishing, where V cm^{-1} is more widely adopted since relatively larger voltage gradients are used.

2.7 Concluding discussion

The current paper defines key aspects relevant to marine electrotrawling and the use of appropriate abbreviations/symbols and units. The aim was to provide information on the physiological effects on organisms and physical parameters of electrical (pulse) stimulation, explain associated electrical parameters, and provide best-practice recommendations for presenting and publishing results in this field. Together these guidelines will eliminate unclear or contradictory use of waveform parameters and harmonise descriptions and terminology. We hope they will enable qualitative and transparent discussions and comparisons, and facilitate accurate repetition of electrofishing experiments. In addition, these guidelines will provide a concise and comprehensible manual for those not familiar with this topic.

The need for this reference work was expressed by the Working Group on ELECtrical TRAwling (WGELECTRA) of the International Council for the Exploration of the Sea (ICES). WGELECTRA recommends these guidelines as a consistent approach to better communication standards in electrofishing, and pulse trawling in particular. In addition, we believe that these guidelines are also useful for freshwater electrofishing studies and hope it will promote closer collaboration between these, currently insufficiently intertwined, research fields. Hence, this summary aimed to incorporate existing terms and abbreviations from both freshwater and marine electrofishing.

2.8 Statements

2.8.1 Funding

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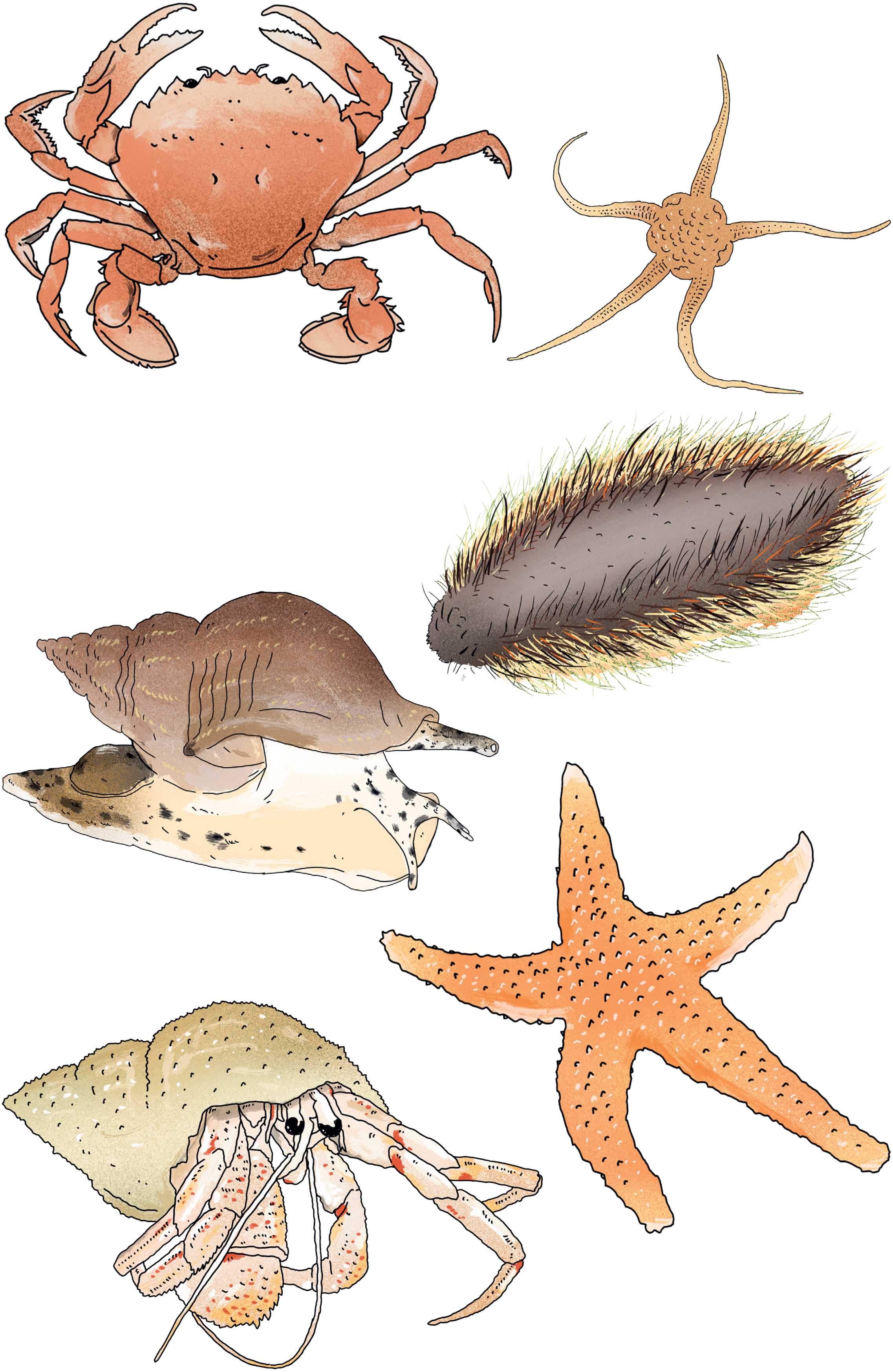

Chapter 3

Effects of electrical pulse stimulation on behaviour and survival of marine benthic invertebrates

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Abstract

Electrical pulse trawling is an alternative to conventional beam trawling for common sole (*Solea solea*), with the potential for higher revenues and less impact on the marine ecosystem. Concerns exist, however, that benthic invertebrates might be seriously affected by pulse fishing. Even if direct injuries and mortality were limited, changes in behaviour might compromise their survival, with potentially large impacts on food webs. Here, we investigate effects of electrical pulses on locomotion behaviour and 14-days survival of six invertebrate species from four phyla that may encounter pulse fishing gears. Electrical stimulation consisted of a Pulsed Bipolar Current at 200 V m^{-1}, 30 Hz, 0.33 ms pulse width, and 3 s duration. We quantified species-specific behaviours before, during, and after electrical stimulation and compared these to a non-exposed control group. Responses during stimulation varied from no visible effect (echinoderms) to squirming (sea mouse) and retractions (whelk and crustaceans). Within 30 s after stimulation, all animals resumed normal behavioural patterns, without signs of lasting immobilisation. Starfish, serpent star, whelk and sea mouse showed no change in movement patterns after stimulation, whereas flying crab and hermit crab showed significant changes in activity that were indicative of increased shelter behaviour. For none of the species, survival at 14-days after stimulation was negatively affected. These findings suggest that changes in locomotion behaviour due to electrical stimulation as used in pulse trawling are unlikely to substantially compromise survival of the investigated species.

Keywords: animal behaviour, benthic impact, bycatch species, electrical pulse fishing, locomotion activity, mortality, North Sea, righting reflex

3.1 Introduction

Bottom trawling for benthic species makes up a large proportion of global marine capture fisheries (Amoroso *et al.*, 2018; Cashion *et al.*, 2018; Watson and Tidd, 2018). Different types of bottom trawls have been developed depending on seabed habitats and target species (Watson *et al.*, 2006; Eigaard *et al.*, 2017). In northwestern European waters, beam trawls with tickler chains have conventionally been used to catch flatfish (Rijnsdorp *et al.*, 2008; Lescrauwaet *et al.*, 2013; Eigaard *et al.*, 2016; van der Reijden *et al.*, 2018). The mouth of the net is held open by a metal beam which is attached to two lateral 'shoes' that are pulled across the seabed, although fishermen nowadays may replace the beam and shoes by a hydrodynamic wing (Rijnsdorp *et al.*, 2020a). To chase flatfish out of the sediment and into the following net, chains are transversally pulled through the seabed in front of the ground rope. This type of trawling is frequently criticized for poor selectivity (Kaiser and Spencer, 1995; Depestele *et al.*, 2014; Bayse *et al.*, 2016), for large disturbance of the benthic ecosystem (de Groot, 1984; Lindeboom and de Groot, 1998; Paschen *et al.*, 2000; Kaiser *et al.*, 2006; Depestele *et al.*, 2016), and for high fuel consumption (Poos *et al.*, 2013).

As an alternative to tickler chains for chasing fish from the seabed, fishermen targeting common sole (*Solea solea*) in the southern North Sea have introduced electrical pulse gears that can be installed on the same fishing vessels (Soetaert *et al.*, 2015a). These so-called pulse trawlers use pulsed electric fields to induce involuntary muscle contractions in the fish which chase them out of the sediment and immobilize them in front of the nets (Soetaert *et al.*, 2019). The electrode arrays are dragged over the sediment in parallel with the towing direction and are typically towed at a lower speed than tickler chain gears (Rijnsdorp *et al.*, 2020a). Pulse trawling is permitted under temporary derogations of the European Union's prohibition to catch marine organisms using electric current (Haasnoot *et al.*, 2016; Soetaert *et al.*, 2019). Compared to beam trawlers with tickler chains or chain mats (Rijnsdorp *et al.*, 2008; Eigaard *et al.*, 2016), pulse trawlers have several advantages, including increased selectivity (van Marlen *et al.*, 2014; Poos *et al.*, 2020) and discard survival (van der Reijden *et al.*, 2017), and reduced seabed damage (Depestele *et al.*, 2016, 2019; Tiano *et al.*, 2019; Rijnsdorp *et al.*, 2020a) and bycatch rates (van Marlen *et al.*, 2014). Additionally, higher net revenues (Batsleer *et al.*, 2016), resulting from higher catch efficiencies for sole (Poos *et al.*, 2020) and 30–50% lower fuel consumption (van Marlen *et al.*, 2014; Poos *et al.*, 2020), make pulse trawling a promising alternative with potentially less impact on the ecosystem.

Despite these advantages of pulse gear over tickler chains, concerns exist regarding possible negative impact of electrical stimulation on invertebrates (ICES, 2018; Quirijns *et al.*, 2018). Electrotrawling for sole exposes benthic marine invertebrates to high electric field strengths near the electrode arrays (de Haan *et al.*, 2016; de Haan and Burggraaf, 2018), which might compromise survival. Previous studies have shown that exposure to electrical pulses as used in electrotrawling for sole causes no, or very limited, internal injuries or direct mortality in marine benthic invertebrates (Smaal and Brummelhuis, 2005; van Marlen *et al.*, 2009; Soetaert *et al.*, 2015b, 2016). However, as invertebrate species are generally not retained, indirect mortality may occur after a trawling event due to increased predation risk related to injuries or behavioural changes (e.g., Kaiser and Spencer, 1994; Evans *et al.*, 1996; Collie *et al.*, 2000; Chícharo *et al.*, 2002). Increased mortality due to fishing efforts may disturb food web structures and hence indirectly affect the population dynamics of a wider range of species (e.g., Kaiser *et al.*, 2002; Hiddink *et al.*, 2011; van Denderen *et al.*, 2013; Collie *et al.*, 2017). It is unknown to what extent electrical stimulation affects invertebrate behaviour. Here, we especially investigate effects on locomotion behaviour, which could increase predation susceptibility after a trawling event (Murray *et al.*, 2016), leading to indirect mortality.

Due to the large variety in neuromuscular and musculoskeletal systems of marine benthic invertebrates (e.g., Cattaert and Edwards, 2017; Hartenstein, 2017; Kristan, 2017), an electrical stimulus may have different effects on locomotion behaviour. To assess the potential impact on locomotion performance in benthic invertebrates, we studied the effects in six species: common starfish (*Asterias rubens*), serpent star (*Ophiura ophiura*), common whelk (*Buccinum undatum*), sea mouse (*Aphrodita aculeata*), common hermit crab (*Pagurus bernhardus*), and flying crab (*Liocarcinus holsatus*). These species represent four different phyla, inhabit areas trawled by pulse trawlers (e.g., Witbaard *et al.*, 2013; ICES, 2018), and have been previously used as model species.

To measure the impact of electrical pulses on behaviour, we measured species-specific locomotion behaviour before and after electrical stimulation and we compare the results to those for a non-exposed control group. In addition, we studied the acute behaviour during electrical stimulation and the recovery from any acute responses thereafter. Species-specific locomotion behaviours were chosen that may indicate prolonged changes related to predation risk. These behaviours include righting reflexes and locomotion activity such as walking and burying. Righting reflexes have been used as overall health and stress indicators in echinoderms (Lawrence and Cowell, 1996; Canty *et al.*, 2009), and as stress indicator in gastropods, where delayed righting duration may increase predation risk (Ramsay and Kaiser, 1998). Burying activity has been used as bioassay for predation risk in annelids (Schaum *et al.*, 2013). In crustaceans, general activity scores have been used as indicators for predation-risk-related behaviour (Ejdung, 1998). By recording differences in righting duration and locomotion activity between control and exposure groups, we effectively detect responses to electrical stimulation. Particularly, increased righting duration and prolonged immobility after stimulation would be indicative of compromised survivability. Finally, we assessed animal survival at 14 days after the behavioural assessment to expand on previous experiments (Smaal and Brummelhuis, 2005; van Marlen *et al.*, 2009; Soetaert *et al.*, 2015b, 2016).

3.2 Materials and methods

3.2.1 Experimental procedure

Animals were acclimated to the laboratory housing at least two weeks prior to experimentation and were fed two-to-three times per week (**Supplementary material 3.1**). The experimental procedure consisted of a pre-treatment measurement of species-specific behaviour, a stimulation period of 3 s, with electrical pulses turned on (exposure group) or turned off (control group), a 30 s recovery period, followed by measuring post-treatment species-specific behaviour similar to the pre-treatment measurement. Animals were transferred from the housing tank to the experimental setup while submerged in a transportation cup. The pre-treatment behavioural assessment started directly after placing the animal in the setup. To start the post-treatment measurements in a similar way, animals were also placed in the transportation cup after the 30 s recovery period.

Pre- and post-treatment measurements were adapted to the relevant behavioural repertoire of each species. An overview of the species-specific experimental procedure, including the number of animals, is provided in **Table 3.1**. Experiments were performed under well-controlled laboratory conditions in one of two identical setups, for one individual at the time. Waiting times between pre-treatment behavioural measurements, the treatment, and post-treatment measurements were minimised and similar in the control and exposure group. Animals were randomly assigned to the exposure or control group. After completing the behavioural measurements, wet mass and body length were measured (**Supplementary material 3.2**) and the animals were transferred to the housing tanks to monitor survival for 14 days.

3.2.2 Experimental animals and housing facilities

All animals were caught using short (~20 min) fishing hauls with a 4-metre beam trawl and collected by scientists of the Flanders Research Institute for Agriculture, Fisheries and Food (ILVO). Common starfish, serpent star, common hermit crab, and flying crab were collected off the Belgium coast. Common whelk and sea mouse were collected off

Table 3.1. Overview of species, number of animals per treatment group, and behavioural response measurement types per species. In addition to pre- and post-treatment response measurements, acute behavioural responses were scored during stimulation and in the following 30 s (not shown). In common starfish and serpent star, walking duration was measured directly after the fifth pre-treatment righting event (i.e., during treatment and extending in the post-treatment period), because these species lacked an acute behavioural response. The number of consecutive behavioural response measurements (*nx*; i.e., number of events) is provided in parentheses. After the behavioural response measurements, survival was monitored for 14 days (not shown).

Species	**Number of animals**		**Behavioural responses**				
	Control	**Exposure**	**Pre-treatment**		**During treatment**	**Post-treatment**	
Common starfish	44	41	Righting duration (5x)		← Walking duration (1x) →		Righting duration (5x)
Serpent star	21	21	Righting duration (5x)		← Walking duration (1x) →		Righting duration (5x)
Common whelk	46	41	Righting duration (1x)	Duration until start of burying or crawling duration (1x)		Righting duration (1x)	Duration until start of burying or crawling duration (1x)
Sea mouse	44	42	Righting duration (1x)	Duration until start of burying or walking duration (1x)		Righting duration (1x)	Duration until start of burying or walking duration (1x)
Common hermit crab	43	43	Righting duration (1x)	Locomotion activity (1x)		Righting duration (1x)	Locomotion activity (1x)
Flying crab	46	44	Locomotion activity (1x)			Locomotion activity (1x)	

the English coast. Animals without visible injuries were selected and transported to the ILVO laboratory facilities in Ostend (Belgium) within 3 hours after docking.

Each species was housed separately in tanks containing aerated natural seawater in a closed circulation system. Water quality parameters were monitored daily, including ammonium, nitrite, and nitrate (MQuant) and dissolved oxygen concentration and saturation, temperature, salinity, and conductivity (YSI Pro2030). Ammonium levels were always below 0.5 mg L^{-1} and salinity varied between 33.0–34.3. Water was partially changed when nitrite or nitrate levels exceeded 0.05 and 25 mg L^{-1} respectively. Starfish and whelk were kept in a room with artificial lighting consisting of a 20 W white LED floodlight (Bailey) at a 12:12 L:D regime. The other species were kept in a room with natural lighting conditions (October–December). Within this period, water temperatures partially followed outside temperatures and varied between 12.6–16.4 °C, as the areas were not climate controlled. However, measurements for each species were conducted in short periods, and measurements for the control and exposure groups were balanced, thus limiting a possible effect of temperature.

All animals were housed in tanks with calibrated sediment (0.2–0.5 mm grain size) to allow for natural burying behaviour (e.g., van Dam, 1940; Dyer *et al.*, 1982). To minimise stress and damage resulting from agonistic behaviour (Lee and Seed, 1992; Ramsay *et al.*, 1997), housing for hermit and flying crab was enriched with shelters, and crabs were transferred to individual containers one week prior to the experiment (same as used during survival monitoring; see below).

3.2.3 Experimental setup

Each experimental setup (**Figure 3.1A**), consisted of a glass tank with a 5 cm layer of levelled, calibrated sediment and two, plate-shaped stainless-steel electrodes placed in the width of the tank at 43 cm apart, and 1 cm from the tank's bottom. The gaps between the electrodes and glass side walls were closed off with PVC sheets to prevent animals from leaving the measurement area. The plate-shaped electrodes created a homogeneous electric field, minimising exposure variability (Soetaert *et al.*, 2015b). Each day, the tanks were filled with water from the housing tanks of the animals that would be used that day. No filter was connected to the experimental tanks, but water was aerated after each individual measurement session. Water quality parameters, including temperature were monitored at the end of a measurement day and were found to remain stable and similar to the housing tanks.

We used two Casio Exilim Pro EX-F1 cameras (1280 x 720 px, 30 fps) per tank to record the behaviour, one with a top view and one with a front view of the measurement area. Two floodlights above the front camera illuminated the setup. A transparent Plexiglas tray on top of the water prevented image distortions due to the water surface. Digital clocks (Technoline WS 8005 and Fisher Scientific) and a pulse stimulus indicator light were placed in view of the cameras for precise timing information. To minimise external disturbances, black curtains were placed around and above each experimental setup.

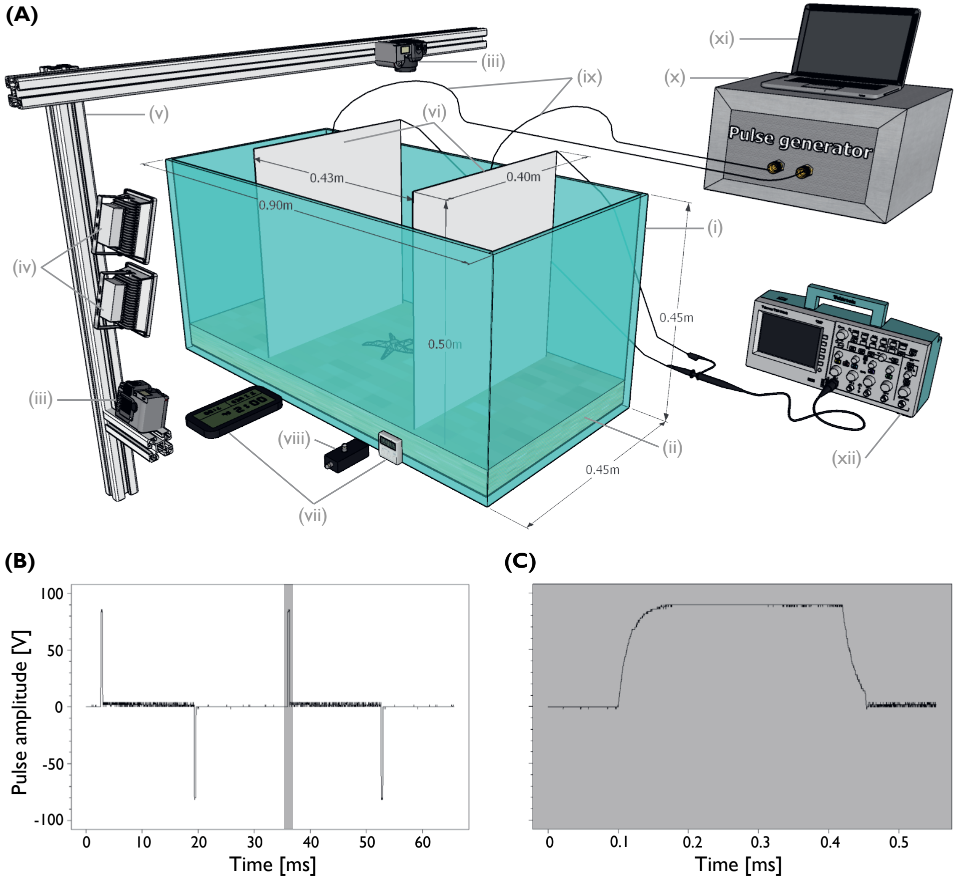

Figure 3.1. (A) Schematic illustration of the experimental setup: (i) glass tank, (ii) sediment, (iii) cameras, (iv) floodlights, (v) aluminium frame, (vi) plate-shaped stainless-steel electrodes, (vii) digital clocks, (viii) pulse stimulus indicator light, (ix) cables connecting electrodes with output connectors of (x) the pulse generator, (xi) computer to control the generator, and (xii) oscilloscope (Tektronix TDS 1001B). **(B)** Oscilloscope data showing two pulse cycles at 30 Hz (out of 90 cycles in total) of which the single electrical pulse in the grey shading is enlarged in **(C)**. Pulse waveform is 30 Hz PBC (*PW* = 0.33 ms, *PB* = 16.34 ms) (Soetaert *et al.*, 2019).

The electrical stimulus, generated by a high-power laboratory pulse generator (LPG, EPLG bvba, Belgium) consisted of a rectangular-shaped Pulsed Bipolar Current (Soetaert *et al.*, 2019) at a frequency of 30 Hz and pulse width of 0.33 ms (**Figure 3.1B**, **C**) These pulse parameters are similar to those used in a HFK PulseWing system targeting sole (Soetaert *et al.*, 2014, 2019; pers. comm. Harmen Klein Woolthuis of HFK Engineering B.V.). We used a pulse exposure duration of 3 s, which is about twice the duration animals would experience for commercial fishing gears with 4 m long electrode arrays (de Haan *et al.*, 2016; Soetaert *et al.*, 2019) towed at about 5 knots (van Marlen *et al.*, 2014; Depestele *et al.*, 2019; Poos *et al.*, 2020; Rijnsdorp *et al.*, 2020a). The electric field strength was set to 200 V m^{-1} (V_{pk} on electrodes = 86 V), which equals the field strength at about 3 cm distance to commercial electrodes that are 40 cm apart (de Haan *et al.*, 2016). A feedback system controlled the voltage, which was additionally monitored with an oscilloscope. At the start of a treatment, the animals were located upright in the middle of the experimental area, on top of the sediment. For species that needed repositioning, all animals were handled equally.

3.2.4 Behavioural responses

For all species, except flying crab, species-specific behaviours included righting duration as relevant locomotion behaviour, where increased righting times may reveal negative effects of pulse stimulation. Single pre- and post-treatment rightings were measured to maximise the number of specimens per species, except for echinoderms, where we repeated rightings 5 times in accordance with variations in righting duration reported by Lawrence and Cowell (1996). Pilot measurements indicated low variability in righting duration for the other species. Because rightings sometimes take very long, or may be postponed indefinitely, one has to set a time limit to righting measurements. Time limits were species-specific and based on pilot measurements and, if available, literature data (Lawrence and Cowell, 1996; Davies *et al.*, 1998; Ramsay and Kaiser, 1998; Canty *et al.*, 2009). In all cases, the limits were at least 3 standard deviations above the mean righting duration. If an animal passed the time limit during the pre-treatment measurement (which was identical for exposure and control groups), we removed the animal from the experiment (numbers given in the results). These animals could not have shown increased post-treatment righting durations, and the same rule was applied for control and exposure groups. These exclusions were therefore unlikely to reduce a potential effect, and allowed us to increase the sample size and gain statistical power. Post-treatment time-outs were scored as missing data, and we performed an extended analysis to test the robustness of the statistical tests for different ‘potential’ durations (see results). Species-specific behaviours, such as walking/crawling and burying started after the pre- and post-treatment righting measurements. However, for starfish and serpent star that both lacked a clear response to stimulation, walking duration was measured starting immediately after the fifth righting event when the treatment was provided (i.e., only once), to increase sensitivity for detecting a potential effect.

To measure righting duration for starfish and serpent star, animals were picked up by hand from an upright orientation, with equal arm orientations, and were placed upside down on the sediment. Righting duration was defined as the time between the aboral surface touching the sediment and the ambulacra of all five arms in contact with the sediment (Canty *et al.*, 2009). Starfish were given 17 min to right during each of the first five righting events. For starfish and serpent star walking duration was quantified by the time to move from the centre of the tank, directly after the fifth righting event, to the tank wall or electrode. We also scored when animals started burying in the sediment, in which case walking duration was a missing value.

For whelk and sea mouse, righting duration was measured once before and after treatment, in a procedure similar to that for the echinoderms. The animal was hand-released with the shell or dorsal side facing down and righting duration was measured from touching the sediment, until the complete foot or ventral side was in contact with the sediment (Fong *et al.*, 2017). The time limits for rightings of whelk and sea mouse were set to 15 min and 10 min respectively. If animals passed the time limit in the post-treatment assessment they were manually righted for the subsequent behavioural assessment. To quantify locomotion behaviour for whelk and sea mouse we scored how many started burying and we measured (i) the time until the start of burying or (ii) the time to reach a wall if they did not start to bury (crawling/walking duration). Complete burying duration could not be quantified as whelk and sea mouse often remained partly buried (van Dam, 1940; Himmelman and Hamel, 1993). We also scored when animals remained stationary on the sediment after righting, in which case the duration until the start of burying and crawling/walking duration was a missing value. In whelk, this occurred in one control and two exposure group specimens before treatment and two control and three exposure group animals after treatment. The post-treatment specimens that remained stationary include the three animals that were also stationary in the pre-treatment measurement.

For hermit crab we measured righting duration once before and once after treatment, in a procedure similar to that for whelk and sea mouse. Righting duration ended when all legs were in contact with the sediment and the shell was rotated to the normal position. We also quantified locomotion activity in a 500 s period after the righting reflex. Locomotion activity was quantified from the top camera footage, using an automated tracking procedure programmed in `Python` (Python Software Foundation, n.d.) in combination with `OpenCV`. The tracking algorithm provided a measure of the area changed from frame to frame, and of the number of objects corresponding to these changes (**Supplementary material 3.3**). The former is more sensitive to whole-body movements, whereas the latter is also highly sensitive to movements of body extremities in the absence of whole-body movements. For flying crab, righting duration could not be measured and we only quantified locomotion activity, as described for the hermit crab.

Apart from crustacean locomotion activity, behavioural responses were scored in real-time. In case of doubt or missing values, the behaviours were scored from recorded videos.

3.2.5 Survival

After behavioural response measurements, animals were individually placed in custom-built containers and returned to their housing tanks (**Supplementary material 3.4**). Survival monitoring was based on Kaiser and Spencer (1995) and performed daily on weekdays for 14 days. During survival monitoring, animals were not fed to emphasise possible vitality differences.

3.2.6 Statistical analyses

The effect of treatment on righting duration, on walking/crawling duration, on the duration until the start of burying, and on locomotion activity (crabs) was analysed by fitting a linear mixed effect model by Restricted Maximum Likelihood. Each model included treatment (i.e., control and exposure), event (i.e., pre- and post-treatment measurements), as well as their interaction as fixed effects. Body mass and length were included as additional fixed effects to exclude a potential effect of body size differences between control and exposure groups on the measured behaviours. We include both body mass and length to account for changes in shape when animal size differs. Individual identity was included as a random effect to correct for pseudo-replication and for between-individual variation irrespective of fixed effects. Comparisons between the control and exposure group within each event were subsequently performed using a multiple comparison procedure. Because we used a species-specific design, models were applied to each species separately. Although body mass and length are collinear, this is irrelevant since we do not aim to disentangle these predictors, but merely wish to discount them in estimating the effect of electrical pulse treatment. Because we correct for body mass and length in the multiple comparison procedure, estimated treatment effects are independent of body size. Since walking duration for echinoderms was measured once, the potential effects were estimated in linear models (no random effects) using Maximum Likelihood. Similar to the linear mixed effect models, treatment, body mass, and length were included as fixed effects. To meet the assumptions of normality and homoscedasticity, we performed a visual assessment for optimal data transformation. All species-specific behaviour data were ln-transformed, except for flying crab, where we applied a power transformation because in four instances a zero value was present in the dataset (animals remained stationary). The power parameter, lambda, was optimised for the area changed and for the number of objects separately (0.265 and 0.357, respectively). See **Supplementary material 3.5** for additional information on the statistical methods of the behavioural measurements.

The effect of electrical exposure on survival at 14 days after the behaviour measurements was assessed by fitting a generalized linear model by Maximum Likelihood and a logit link for the quasi-binomially distributed response, with treatment, species (i.e., common starfish, serpent star, common whelk, sea mouse, common hermit crab, and flying crab), and their interaction as fixed effects. Similarly to behavioural models, effects of body mass and length were taken into account. Species-specific comparisons of survival between the treatments were subsequently performed using a multiple comparison procedure. We used the quasi-binomial distribution because data were found to be underdispersed ($\varphi = 0.39$) when we used a binomial distribution. We compare results for the two distributions and further motivate the choice for the quasi-binomial distribution in **Supplementary material 3.6**.

All statistical analyses were performed in `R` v3.6.1 (R Core Team, 2019). Visual assessment of the optimal data transformation for the behavioural models was performed using the `symbox` function from the `car` package (Fox and Weisberg, 2019), mixed models were fitted using the `lme4` package (Bates *et al.*, 2015), significance tests for the fixed effects were performed with `lmerTest` (Kuznetsova *et al.*, 2017), and multiple comparison procedures were performed with `multcomp` (Hothorn *et al.*, 2008). *P*-values were adjusted for multiple testing using the single-step method.

3.3 Results

3.3.1 Behavioural responses

Starfish and serpent star continued their normal behaviour during electrical stimulation, without signs of immobilisation or agitation. Starfish and serpent star either continued walking or burying, where starfish generally walked and serpent star more often buried.

Pre-treatment righting durations for all starfish were 2.47 ± 1.85 min (mean ± SD), with no clear trend, except for a slightly larger variability in the first righting (**Figure 3.2A**). Ten starfish (out of 95 animals in total) passed the 17 min time limit for righting in the pre-treatment period. Post-treatment righting times were similar to the pre-treatment times (2.25 ± 1.57 min and 2.31 ± 1.17 min for control and exposure group respectively). Serpent star righted within several seconds with limited variation (**Figure 3.2B**). No significant differences were found between treatments in righting duration in any of the righting events, for starfish nor for serpent star (see **Supplementary material 3.5** for

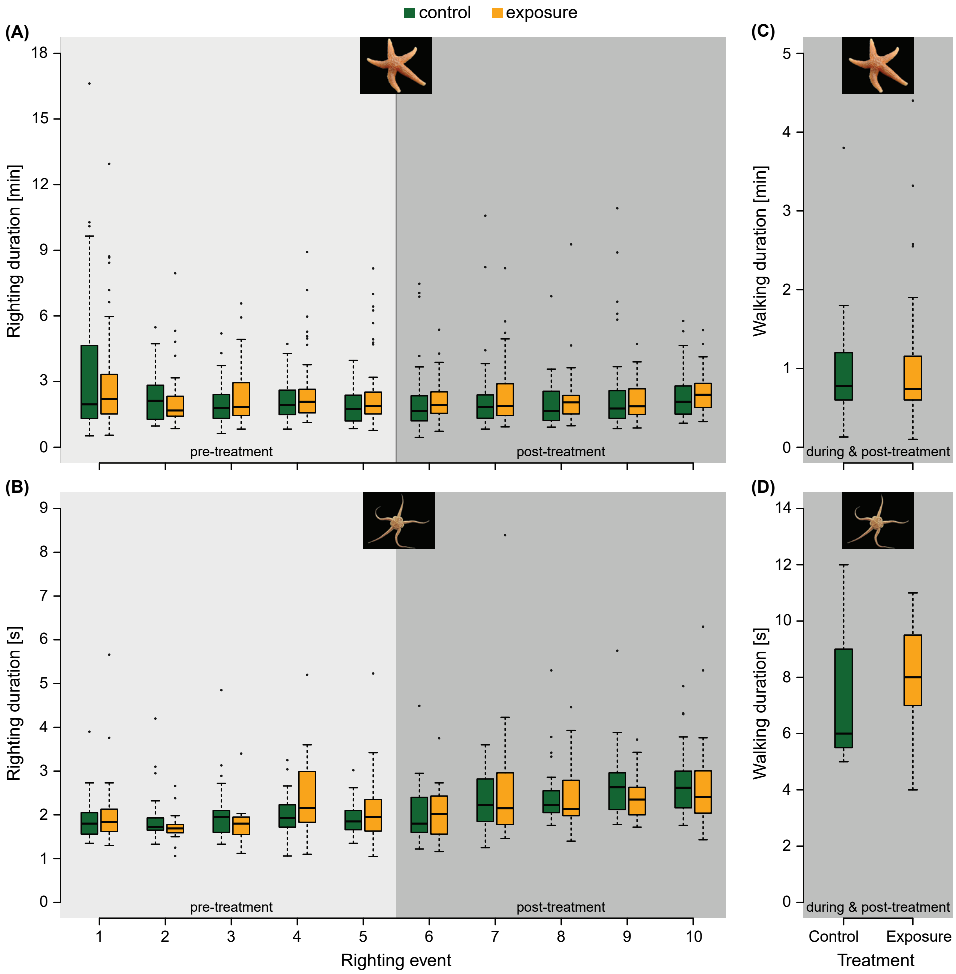

Figure 3.2. Pre- and post-treatment response measurements of locomotion behaviours in starfish and serpent star for the control group (green) and exposure group (orange). **(A)** Righting duration of starfish ($n_{control}$ = 44 in righting event 1 to 8, $n_{control}$ = 43 in righting event 9 and 10, $n_{exposure}$ = 41). **(B)** Righting duration of serpent star ($n_{control}$ = 21, $n_{exposure}$ = 21 in righting event 1 to 9, $n_{exposure}$ = 20 in righting event 10). **(C)** and **(D)** Walking duration of starfish ($n_{control}$ = 42, $n_{exposure}$ = 40) and serpent star ($n_{control}$ = 8, $n_{exposure}$ = 16) respectively. Photographs by © Hans Hillewaert, ILVO.

additional information on statistical output of the behavioural measurements). More exposed serpent star started walking instead of burying (76.2% versus 38.1% for the control). Walking durations were neither significantly different between the control and exposed specimens for starfish ($t_{78} = 0.233$, $p = 0.816$; **Figure 3.2C**) nor for serpent star ($t_{20} = 1.389$, $p = 0.180$; **Figure 3.2D**).

All whelk retracted, at least partly, in their shell during electrical stimulation. Responses immediately after electrical exposure were variable: one specimen remained retracted for the full 30 s, 14.6% exhibited escape type of movements by contorting the foot, but most animals showed limited locomotion activity. During or immediately after stimulation 63.4% of the animals ejected a white substance, presumably related to reproduction. The percentages of whelk, in control and exposure group respectively, that remained stationary (60.9% and 63.4%), crawled (37% and 22%), or buried (2.2% and 4.9%) were similar. Pre-treatment righting durations for all whelk were 4.67 ± 3.16 min. Sixteen whelk (out of 103 animals in total) passed the 15 min time limit for righting in the pre-treatment period. Post-treatment righting durations were shorter, in both control

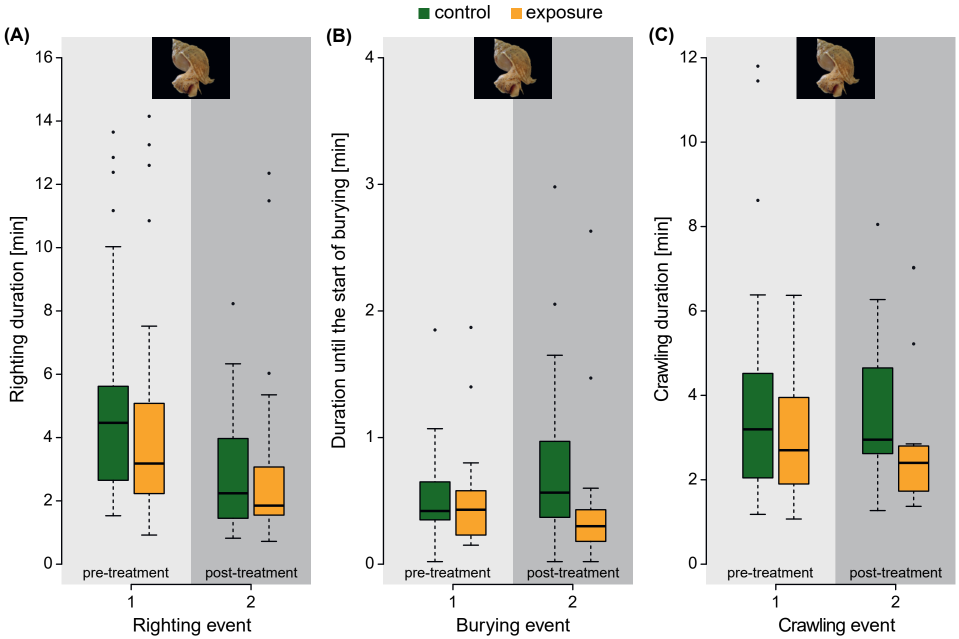

Figure 3.3. Pre- and post-treatment response measurements of locomotion behaviours in whelk for the control group (green) and exposure group (orange). **(A)** Righting duration of whelk ($n_{\text{pre\&post,control}} = 46$, $n_{\text{pre\&post,exposure}} = 41$), whereafter each righting event, whelk could either start burying or crawl to the side of the setup, or remain stationary. **(B)** Duration until the start of burying of whelk ($n_{\text{pre,control}} = 25$, $n_{\text{pre,exposure}} = 21$, $n_{\text{post,control}} = 26$, $n_{\text{post,exposure}} = 17$). **(C)** Crawling duration of whelk ($n_{\text{pre,control}} = 20$, $n_{\text{pre,exposure}} = 18$, $n_{\text{post,control}} = 18$, $n_{\text{post,exposure}} = 21$). Photographs by © Hans Hillewaert, ILVO.

(2.81 ± 1.63 min) and exposed group (2.74 ± 2.40 min) (**Figure 3.3A**), but differences between the control and exposed group were not significant ($z = -0.973$, $p = 0.527$). After electrical stimulation, the time until the start of burying seemed lower for the exposed (29.5 ± 37.2 s) than for the control specimens (47.7 ± 38.6 s), but the difference was not significant ($z = -1.701$, $p = 0.162$; **Figure 3.3B**). Slightly more exposed whelk started crawling instead of burying (51.2% versus 39.1% for the control). Crawlers reached the wall in 3.64 ± 1.74 min (control) and 2.74 ± 1.60 min (exposed group). This difference was also not significant ($z = -1.685$, $p = 0.170$; **Figure 3.3C**).

About half of the sea mouse (47.6%) showed a squirming type of movement during stimulation. The remainder either kept walking (2.4%) or burying (11.9%), or showed no obvious response. In the 30 s after stimulation, no major differences between treatment groups were observed. Exposed sea mouse showed a slightly higher tendency to start walking (38.1% versus 15.9% for the control group), and started burying less frequently (35.7% versus 50%). The other animals remained stationary (26.2% for exposed and 34.1% for control animals). Pre-treatment righting times for all animals were 1.19 ± 1.27

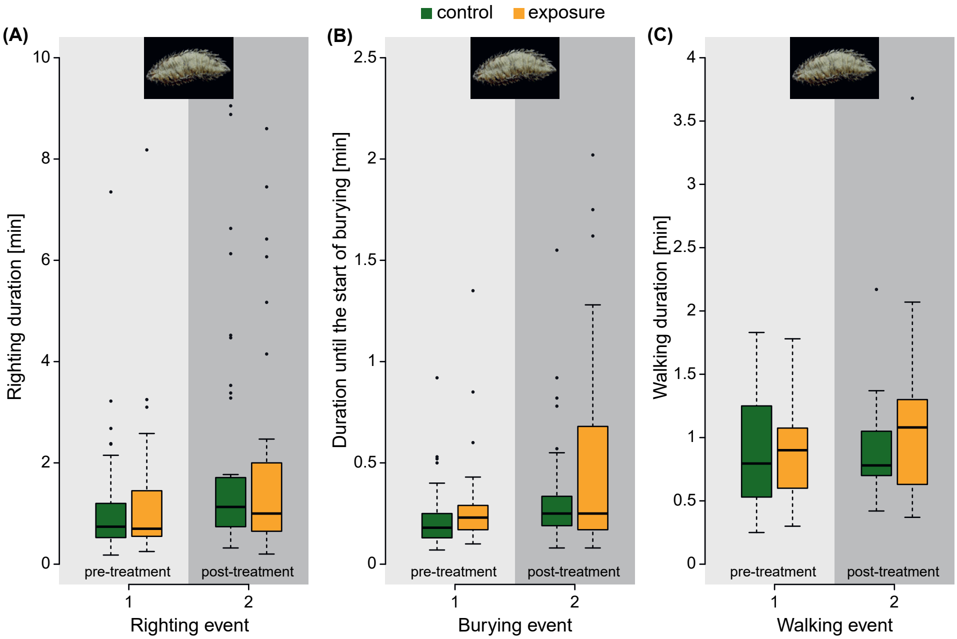

Figure 3.4. Pre- and post-treatment response measurements of locomotion behaviours in sea mouse for the control group (green) and exposure group (orange). **(A)** Righting duration of sea mouse ($n_{pre,control} = 44$, $n_{pre,exposure} = 42$, $n_{post,control} = 39$, $n_{post,exposure} = 34$), whereafter each righting event, sea mouse could either start burying or walk to the side of the setup. **(B)** Duration until the start of burying of sea mouse ($n_{pre,control} = 30$, $n_{pre,exposure} = 23$, $n_{post,control} = 35$, $n_{post,exposure} = 25$). **(C)** Walking duration of sea mouse ($n_{pre,control} = 14$, $n_{pre,exposure} = 19$, $n_{post,control} = 9$, $n_{post,exposure} = 17$). Photographs by © Hans Hillewaert, ILVO.

min (**Figure 3.4A**). Two sea mouse (out of 88 animals in total) passed the 10 min time limit for righting in the pre-treatment period. In addition, for 5 control and 8 exposed animals post-treatment values were missing due to passing the time limit in the post-treatment period. Because here extended righting times indicate a potential effect, these missing data potentially bias our results. To check this, we included them by assigning a value of 11 min (time limit plus 1) to the missing control animals and increasingly larger values for the exposed group. For values 10 times greater than the time limit, the effect of treatment was still non-significant ($z = 2.132$, $p = 0.062$). This factor of 10 (i.e., 100 min) corresponds to a highly unlikely value of 45 standard deviations (2.17 min) above the mean (1.94 min) of the measured righting durations. Relatively more sea mouse started to bury in the post-treatment period compared to the pre-treatment period, but this effect was similar for the control (79.5% and 68.2%) and exposure group (59.5% and 54.8%). Also, the duration until the start of burying (**Figure 3.4B**) was similar for the control (20.5 ± 17.2 s) and for the exposed specimens (32.2 ± 33.9 s) and did not differ significantly ($z = 1.268$, $p = 0.355$). Animals that ensued walking after righting, took 57.7 ± 29.7 s (control) and 73.0 ± 46.8 s (exposed) to reach the wall (**Figure 3.4C**), which were not significantly different ($z = 1.040$, $p = 0.480$).

Hermit crabs immediately retracted, mostly completely, into the shell upon stimulation. Immediately after stimulation behaviours of exposed and control animals were similar: The majority emerged within 30s and started walking or burying. Most hermit crabs righted within one minute in both treatment groups (**Figure 3.5A**). Post-treatment righting durations were significantly longer for the exposed group compared to the control group ($z = 3.807$, $p < 0.001$). This was due to prolonged retraction durations, because hermit crabs showed variable emerging times. When we scored post-treatment righting duration starting at the moment of emerging from the shell (**Figure 3.5B**), the difference between the control (6.8 ± 13.0 s) and exposed animals (9.1 ± 11.4 s) was not significantly different ($t_{81} = 1.663$, $p = 0.100$; estimated in a linear model using Maximum Likelihood, with treatment, body mass, and length as fixed effects; **Supplementary material 3.5**). Hermit crabs displayed different behaviours during the locomotion activity period, including filtering sediment, walking, and burying. With locomotion activity quantified as the area changed, the exposed group showed significantly reduced activity compared to the control group in the post-treatment period ($z = -2.220$, $p = 0.047$; **Figure 3.5C**). Yet, no significant differences were found when the locomotion activity was expressed as the number of moving objects ($z = -1.483$, $p = 0.223$; **Figure 3.5D**). These measurements indicate that hermit crabs tend to continue normal activity of their extremities, but show reduced whole-body movements.

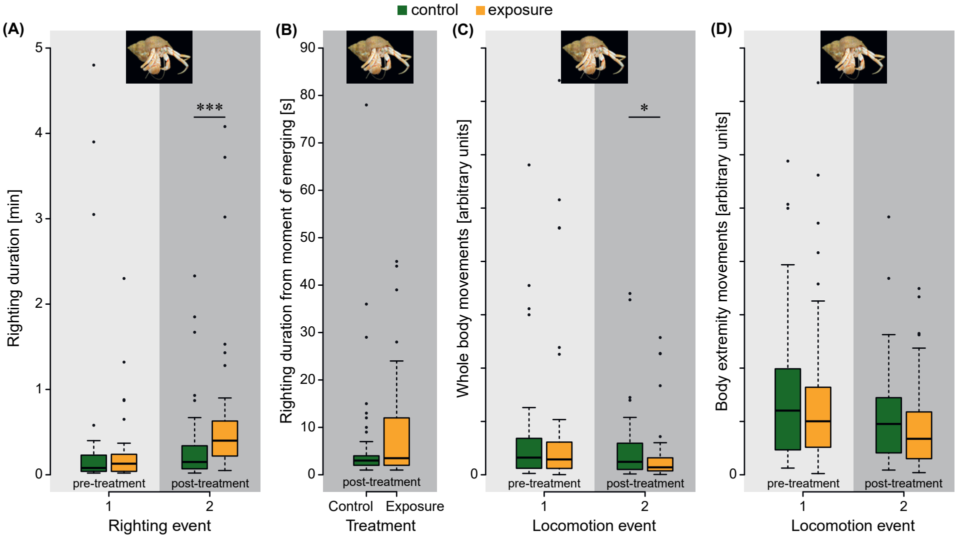

Figure 3.5. Pre- and post-treatment measurements of locomotion behaviours in hermit crab for the control group (green) and exposed group (orange). **(A)** Righting duration of hermit crab as measured from moment of placement on the sediment ($n_{pre\&post,control}$ = 43, $n_{pre,exposure}$ = 43, $n_{post,exposure}$ = 42). The exposed group had a significantly larger righting duration in the post-treatment assessment. **(B)** Post-treatment righting duration as measured from the moment of emerging from the shell ($n_{control}$ = 43, $n_{exposure}$ = 42). After each righting event, locomotion activity was quantified with tracking software using **(C)** the area changed as proxy for whole-body movements and **(D)** the number of objects as proxy for body extremity movements ($n_{pre\&post}$ = 43 in both treatments). Animals in the exposure group had a significantly reduced whole-body movement but not in movements of the body extremities. Significance codes: ***$p \leq 0.001$, 0.01 < *$p \leq 0.05$. Photographs by © Hans Hillewaert, ILVO.

All flying crab withdrew their extremities during stimulation, and thereafter either swam, walked, or ran away immediately, whilst control animals remained stationary or walked slowly. None showed prolonged immobilisation. The exposed group, however, showed significant reductions of locomotion activity in the post-treatment period, both for activity quantified as the area changed ($z = -2.353$, $p = 0.036$; **Figure 3.6A**) and as the number of moving objects ($z = -2.419$, $p = 0.030$; **Figure 3.6B**). Area changed was reduced by a factor of 1.1 for control animals compared to a factor of 2 for exposed animals. Similarly, the number of moving parts was reduced by a factor of 1 for control animals compared to 1.7 for exposed animals. During the locomotion activity measurements animals in both the control and exposed group showed the full range of normal behaviours.

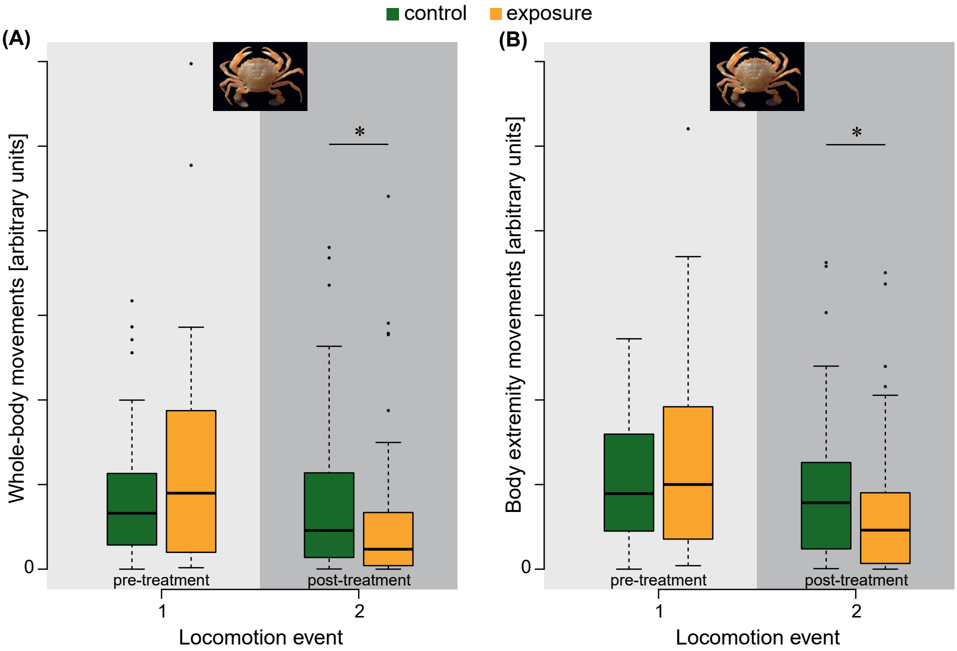

Figure 3.6. Pre- and post-treatment measurements of locomotion activity in flying crab ($n_{\text{pre\&post,control}}$ = 46, $n_{\text{pre\&post,exposure}}$ = 44). **(A)** The area changed as proxy for whole-body movements and **(B)** the number of objects as proxy for body extremity movements. Post-treatment locomotion activity of animals in the exposure group was reduced significantly. Significance code: $0.01 < *p \leq 0.05$. Photographs by © Hans Hillewaert, ILVO.

3.3.2 Survival

All starfish, whelk, and sea mouse survived the 14-days survival period. Serpent star survival of the control and exposure group was 86% and 81% respectively, which did not differ significantly ($z = -0.435$, $p = 0.999$). Hermit crab survival of the control and exposure group was 93% and 91% respectively, which neither differed significantly ($z = -0.800$, $p = 0.963$). A significant difference in survival was found for flying crab, but survival was higher in the exposed group (86%) than the control group (65%) ($z = 3.273$, $p = 0.006$). See **Supplementary material 3.6** for additional information on statistical output of the survival measurements.

3.4 Discussion

Marine benthic invertebrates form a crucial link between primary producers and higher level consumers, hence disrupting invertebrate populations might affect benthic food webs (Covich *et al.*, 2004; Heath, 2005; Sokołowski *et al.*, 2012). To assess potential effects of electrical pulses on locomotion behaviour and survival of invertebrates, we studied six species with diverse neuromuscular and musculoskeletal systems. The selected species, from four phyla, occupy different niches and have different functions in benthic food webs in areas that are likely subjected to pulse trawling.

3.4.1 Behavioural responses

We observed a wide range of acute responses during and immediately after electrical stimulation, ranging from no visible response in echinoderms, to immediate retractions in hermit crabs. Our observations correspond well to previous findings. Smaal and Brummelhuis (2005) and van Marlen *et al.* (2009) also reported absence of an acute response in echinoderms, including the same species, and *Psammechinus miliaris* and *Ophiothrix fragilis*. Other species showed different degrees of escape or defensive behaviours during and immediately after stimulation. In our study about half of the sea mouse squirmed during stimulation, which was not reported by Smaal and Brummelhuis (2005). In *Alitta virens*, another polychaete, a similar response pattern was observed, varying from no response (Smaal and Brummelhuis, 2005) to various intensities of squirming and jerky movements (van Marlen *et al.*, 2009; Soetaert *et al.*, 2015b). Exposed sea mouse showed an increase in walking frequency directly after stimulation, indicating an increased escape response frequency, as sea mouse normally reside (partially) buried in the sediment (van Dam, 1940; Mettam, 1971). Hermit crab retracted and flying crab withdrew their extremities during electrical stimulation, but recovered after stimulation, as also found by Smaal and Brummelhuis, (2005), followed by shelter and escape behaviour. These responses are similar to the tail flip responses in *Crangon crangon* and *Palaemon* spp., and stiffening in *Carcinus maenas* (Smaal and Brummelhuis, 2005; van Marlen *et al.*, 2009; Soetaert *et al.*, 2015b, 2016). Our finding that invertebrates respond differently during stimulation, but that all resume their normal behavioural repertoire mostly within 30 s thus extends similar observations in previous studies.

In common whelk, we observed immediate retraction in their shell, similar to results of Smaal and Brummelhuis (2005) in the same species, and in another marine gastropod, *Tritia reticulata*. However, the observed foot contortions and ejection of a white substance have not previously been reported. Foot contortions are part of the marine gastropod escape response and similar to the behaviour when a potential predator is detected (Harvey *et al.*, 1987; Thomas and Himmelman, 1988; Legault and Himmelman, 1993). Ejection of a white substance, presumably sperm, was observed in 63.4% of the specimens. Experiments were performed in autumn, in the reproductive season of the gonochoric whelk (Heude-Berthelin *et al.*, 2011), and female animals were also observed depositing egg cases in the housing tank. It is unclear to what extent the presumed ejaculation of sperm would compromise whelk reproduction. Moreover, it raises the question whether the female reproductive system could be affected, which might affect egg-capsule depositions.

In all cases, animals resumed normal behavioural patterns within 30 s after stimulation. This, however, does not exclude longer lasting changes in locomotion performance that might compromise survival after a trawling event. To assess such additional effects, we performed quantitative comparisons of behaviours before and after electrical stimulation. These behaviours were chosen to be relevant for each specific species, such as righting reflexes, burying and walking/crawling activity. In particular, we were interested in changes that would increase the risk for predation after a trawling event, such as increased righting times or suppression of escape and shelter behaviours. In general, we found no indications for such effects. Righting duration and locomotion activity were not significantly affected by electrical stimulation in starfish, serpent star, whelk, and sea mouse. However, in serpent star and whelk we found shifts in the frequency of burying and walking/crawling, indicating that stimulation may induce escape behaviours (Himmelman and Hamel, 1993; Sköld, 1998). However, locomotion capacity appeared unaffected as the walking/crawling duration and duration until start of burying were not significantly different between exposed and control groups. Sea mouse, irrespective of treatment, displayed increased burying activities, indicating that their natural behaviour is not disrupted by stimulation. In hermit crab, post-treatment righting durations in the exposure group were significantly longer (**Figure 3.5A**). This, however, was due to increased retraction times, as the animals remained in their shell, resulting in almost complete protection (Kaiser and Spencer, 1995). Increased righting times thus indicate an increase of defensive behaviours that limits rather than increases risk for predation. For hermit crabs, we also found subtle changes in locomotion behaviour that indicate a reduction of whole-body displacements, while maintaining activity patterns related to e.g., feeding.

In flying crab, locomotion activity was significantly reduced, although the animals were obviously capable of immediate escape behaviour after stimulation. Predation risk can induce predation avoidance behaviour (Legault and Himmelman, 1993), by moving to safer habitats (Lima and Dill, 1990). Locomotion activity may reduce in response to predation risk (Lima and Dill, 1990), which decreases the probability of detection by the predator (Ejdung, 1998). Reduced activity of flying crab, due to increased burying activity and remaining stationary along the borders of the experimental area, could indicate that exposed specimens perceived the electrical stimulus as a threat, resulting in shelter behaviour. The shift in behaviour of flying crabs therefore does not necessarily compromise long-term survival.

3.4.2 Survival

We found no negative effect of electrical pulse stimulation on the 14-days survival. The lack of an effect was not due to high variability in survival; in half of the species we found no mortality at all. These findings corroborate with previous findings in starfish, serpent star, whelk, sea mouse, hermit crab, and flying crab (Smaal and Brummelhuis, 2005; van Marlen *et al.*, 2009). For other species (i.e., *Acanthocardia echinata*, *Cerastoderma edule*, *Ensis* spp., *Laevicardium crassum*, *Mytilus edulis*, *Spisula subtruncata*, *Tritia reticulata*, *Ophiothrix fragilis*, *Psammechinus miliaris*, *Carcinus maenas*, *Corystes cassivelaunus*, *Crangon crangon*, *Homarus americanus*, *Palaemon* spp., and *Alitta virens*), variable survival rates after electrical exposure have been reported (Smaal and Brummelhuis, 2005; van Marlen *et al.*, 2009; Soetaert *et al.*, 2015b, 2016). A significant negative effect on survival was found in some species, but only when different stimulus settings were combined in statistical modelling (van Marlen *et al.*, 2009). Soetaert *et al.* (2016) found an effect on 14-days survival, when stimulating repetitively over the course of multiple days, but only compared to one of the two controls. Our results suggest that for many species electrical exposure similar to that in commercial pulse fishing does not compromise survival.

3.4.3 Limitations

Even though our choice of species is limited, our results provide insight into the potential effects of electrical pulses on direct responses, post-treatment behaviour, as well as long-term survival. Obviously, extrapolation of our findings to other species and species groups should be done with caution. Moreover, because we focused on locomotion behaviour, effects on feed intake, growth, and reproduction remained outside the scope of the current study. Future studies could include a wider range of behaviours, and species with other body plans and infauna, as the electric field also penetrates the sediment (de Haan and Burggraaf, 2018).

We used undamaged specimens to minimise variation and therefore focused on effects of electrical stimulation in a healthy population. Injuries are also known to impact behaviour, predator-evasion-responses, and survival (Kaiser and Spencer, 1994, 1995; Ramsay and Kaiser, 1998; Bergman and van Santbrink, 2000; Depestele *et al.*, 2014). Our measurements therefore do not include a potential combined effect of electrical stimulation and injuries. If these effects are of concern, one would need to conduct a much larger experiment, to test the interaction of electrical stimulation and physical condition (e.g., injuries) of animals. Technically this is far more complicated and one should also consider that mechanical disturbance of pulse gears and beam trawl gears may be different (Depestele *et al.*, 2016, 2019; Tiano *et al.*, 2019). Bergman and Meesters (2020) found, for example, that direct mortality of benthic megafauna caused by pulse trawl gear was 43% less than a conventional beam trawl but the difference was not significant. Tiano *et al.* (2020) found no significant differences between the impact on smaller as well as deeper dwelling infauna by PulseWing rigged pulse trawlers and tickler-chain rigged beam trawlers.

To be able to finish a sufficiently large sample, we set a time limit to measurements that might last indefinitely. Time limits were well above the mean righting duration, hence only affect outliers. Animals that passed the limit in the pre-treatment measurements were removed from the experiment. By doing so we intended to increase the sensitivity for finding significant stimulation effects. Not only did it allow for a larger sample size, but animals that already passed the time limit in the pretreatment period could not have shown an increased duration after treatment. Setting time limits focuses the experiment on the average behaviour and may have occluded an effect for very slow animals, but it increased the statistical power and predictability for the bulk of the population, which is arguably more relevant. In our study, missing data were limited, and sensitivity analysis showed that they were highly unlikely to affect our conclusions.

In most cases, we did not find significant differences between the post-treatment control and exposure groups in the quantitative behavioural comparisons. These findings were not limited by sample size, which was sufficient to detect relevant treatment effects (see **Supplementary materials 3.5** and **3.6**). Small, potential differences below the statistical detection threshold, are considered irrelevant, given the time course of mechanical disturbance and sediment resuspension during *in situ* trawling events (Depestele *et al.*, 2016, 2019; Tiano *et al.*, 2019). These additional effects limit visibility, and thus vulnerability, beyond the potentially delayed behavioural response of exposed animals.

We subjected the animals to 200 V m^{-1} in a homogeneous electric field, which equals the electric field strength at about 3 cm next to a fishing gear electrode (de Haan *et al.*, 2016; de Haan and Burggraaf, 2018). Inter-electrode distances for commercial gears are about 40 cm, indicating that the majority of organisms is subjected to substantially weaker stimuli in commercial trawling. In addition, exposures were about a factor of two longer than an animal would experience in commercial trawling. In our experiments, animals were exposed only once to the electrical stimulus. We, therefore, cannot exclude that multiple, repetitive exposures would lead to other effects. Apart from a study by Soetaert *et al.* (2016), that found a limited impact of repetitive exposure on survival, but not on moulting, egg loss, and virus infection in *Crangon crangon*, effects of multiple exposures are unknown. However, the probability of repetitive exposure by commercial trawling is low (ICES, 2018). It therefore seems likely that our stimulus was substantially stronger than what the average population might experience.

Finally, many aspects play a role in assessing the impact of bottom trawling on the ecosystem, including catch efficiency, fishing effort and distribution, physical impact of the gear, seafloor and habitat characteristics, benthic community composition and sensitivity, and food web interactions (e.g., Queirós *et al.*, 2006; Shephard *et al.*, 2010; van Denderen *et al.*, 2014, 2015; Pusceddu *et al.*, 2014; Clark *et al.*, 2016; Depestele *et al.*, 2016; Eigaard *et al.*, 2016; Sciberras *et al.*, 2016; Rijnsdorp *et al.*, 2018; Hiddink *et al.*, 2019). Integrating these aspects into impact assessments of bottom trawling techniques are gaining international momentum (e.g., Eigaard *et al.*, 2017; Hiddink *et al.*, 2017, 2020; Sciberras *et al.*, 2018; Rijnsdorp *et al.*, 2020b; Mazor *et al.*, 2021). Bottom fishing impact assessments, in turn, can support and facilitate fisheries management to reduce fishing effects on ecosystems (e.g., Pikitch *et al.*, 2004; Crowder and Norse, 2008; Suuronen *et al.*, 2012; Kaiser *et al.*, 2016; McConnaughey *et al.*, 2020). Our findings add to a scientific basis for weighing the advantages and disadvantages of electrical pulse fishing compared to other types of bottom fishing.

3.4.4 Conclusion

Survival results nor behavioural results indicate a large detrimental effect of electrical pulses on invertebrates. We found species-specific differences in behavioural susceptibility of benthic marine invertebrates to electrical pulse stimulation. Direct effects were either absent (starfish and serpent star) or squirms (sea mouse), and retractions (whelk, hermit crab, and flying crab), potentially followed by increased escape or shelter behaviour. However, we never observed prolonged immobilisation or abnormal locomotion behaviour in any of the species and locomotion performance was not impaired. Indirect mortality, caused by increased predation susceptibility, is therefore expected to be minimal. Survival was not negatively affected in any species. Our findings provide a strong indication that concerns regarding survivability of invertebrates after electrical pulsing are not supported by scientific evidence.

3.5 Statements

3.5.1 Data availability

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

3.5.2 Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

3.5.3 Author contributions

All authors contributed to the conception and design of the study. PGB and JARN performed the measurements with live animals. MJL programmed and executed the tracking procedure. PGB, JARN, and MJL collected the data, either by scoring real-time or from the video images. PGB performed the statistical analyses and drafted the initial manuscript and figures, with contributions by MS, JARN, and MJL. All authors interpreted the data, discussed the results, contributed to the critical revision of the manuscript and figures, and approved the final version.

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Supplementary material

The supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.592650/full#supplementary-material.

Supplementary material 3.1: Feeding of experimental animals.
Supplementary material 3.2: Body mass and length measurements.
Supplementary material 3.3: Example of activity measurements for crustaceans.
Supplementary material 3.4: The survival experiment.
Supplementary material 3.5: Statistical methods and output of behavioural measurements.
Supplementary material 3.6. Statistical methods and output of survival measurements.

Supplementary material 3.1. Feeding of experimental animals.

Common starfish (*Asterias rubens*) were fed with whole, defrosted blue mussel (*Mytilus edulis*) of which the empty shells were removed within two days after feeding. Serpent star (*Ophiura ophiura*), common whelk (*Buccinum undatum*), common hermit crab (*Pagurus bernhardus*), and flying crab (*Liocarcinus holsatus*) were fed with a mix of cut, defrosted blue mussel meat, common cockle meat (*Cerastoderma edule*), European smelt (*Osmerus eperlanus*), European squid (*Loligo vulgaris*), greater sandeel (*Hyperoplus lanceolatus*), and sprat (*Sprattus sprattus*). Sea mouse (*Aphrodita aculeata*) were fed with live and dead king ragworm (*Alitta virens*), but were never observed feeding. Any uneaten food was removed within two days after feeding.

Supplementary material 3.2. Body mass and length measurements.

Body mass and length measurements were performed after the behavioural measurements and prior to placing the animals in the survival containers **(Supplementary material 3.4)**. Body length was measured to the nearest millimetre, using a calliper (**Figure S3.2**). Wet body mass was measured to the nearest gram (Scout Pro Portable Electronic Balance, Ohaus). The measured mean body mass and length with standard deviations are reported in **Table S3.2** per species and treatment group.

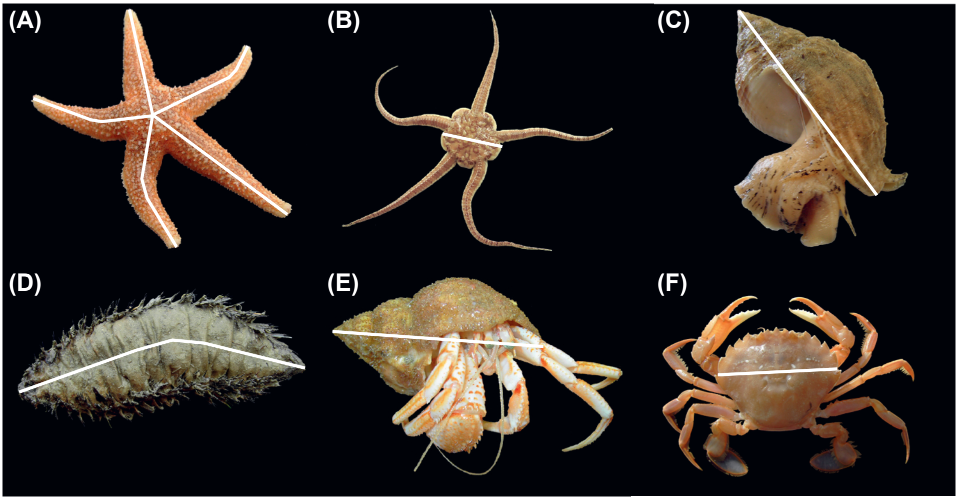

Figure S3.2. The white solid lines indicate over which distance body length was measured. **(A)** In common starfish (*Asterias rubens*) length was quantified by the mean length for all arms. **(B)** In serpent star (*Ophiura ophiura*) disk diameter was used as length parameter. **(C)** In common whelk (*Buccinum undatum*) shell length was used. **(D)** In sea mouse (*Aphrodita aculeata*) length was measured over the midline of the body. **(E)** In common hermit crab (*Pagurus bernhardus*) shell length was used. **(F)** In flying crab (*Liocarcinus holsatus*) carapace width was used as length parameter. Photographs by © Hans Hillewaert, ILVO.

Table S3.2. Mean body mass and length with standard deviations measured per species for each treatment group (i.e., control and exposure).

Species	Number of animals per treatment group		Body mass (mean ± SD) [g]		Body length (mean ± SD) [cm]	
	Control	Exposure	Control	Exposure	Control	Exposure
Starfish	44	41	35.9 ± 13.8	34.7 ± 15.3	6.4 ± 0.9	6.5 ± 1.2
Serpent star	21	21	2.0 ± 0.8	2.3 ± 0.5	1.7 ± 0.2	1.8 ± 0.1
Whelk	46	41	37.9 ± 25.5	40.1 ± 26.6	6.2 ± 1.3	6.3 ± 1.4
Sea mouse	44	42	11.7 ± 4.6	13.3 ± 5.1	6.6 ± 1.2	6.9 ± 1.0
Hermit crab	43	43	10.0 ± 9.0	11.3 ± 11.9	3.8 ± 1.3	3.8 ± 1.5
Flying crab	46	44	12.5 ± 4.5	12.9 ± 4.3	3.6 ± 0.4	3.6 ± 0.4

Supplementary material 3.3. Example of activity measurements for crustaceans.

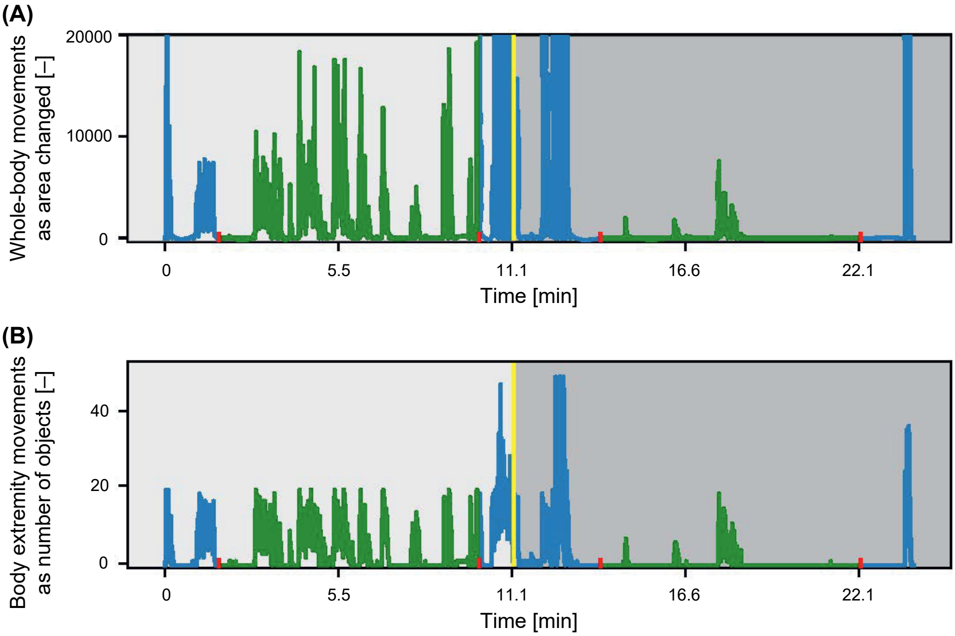

Figure S3.3. Example of the raw data extracted by the tracking software from the images made by the top camera on the experimental setup of a flying crab (*Liocarcinus holsatus*) over time. To automatically track activity we performed a background subtraction, with a dynamically updated background. Next, the difference image was thresholded and we scored the resulting number of detected objects as well as the total area changed from frame to frame. Both measurements are given in arbitrary units, as the absolute value depends on tracking parameters. Parameters were identical for control and exposed animals and for pre- and post-treatment measurements and their relative values were highly consistent for different tracking parameters. Pre- and post-treatment periods are indicated in light and dark grey, respectively. Electrical stimulation occurred at around 11 min, indicated by the yellow vertical line. The sum of the data in green, demarcated by the red tick marks (equivalent to 500 s), is used as locomotion activity proxy for **(A)** whole body moments (area changed) and **(B)** body extremity movements (number of moving objects). In this example, a decrease of locomotion activity after the electrical stimulus is observable by reduced amount of 'green data' in the post-treatment period. Activities indicated in blue were mainly due to handling the animals at the start, around the time of stimulation and at the end of the measurement, and were not taken into account. Handling procedures included adding and removing the animal to and from the setup, moving the animal to the middle of the experimental area after the first 10 min to start stimulation, and temporary removal of the animal after the acute response measurement to level the sediment as was done prior to the pre-treatment measurement period.

Supplementary material 3.4. The survival experiment.

After the behavioural measurements when body mass and length were recorded, animals were transferred to survival containers. Common starfish (*Asterias rubens*), serpent star (*Ophiura ophiura*), sea mouse (*Aphrodita aculeata*), and common whelk (*Buccinum undatum*) were individually placed in custom-build containers made of black plastic mesh (11 x 11 mm) of 25 x 15 cm (height x diameter) **(Figure S3.4A)**. Common hermit crab (*Pagurus bernhardus*) and flying crab (*Liocarcinus holsatus*) were individually placed in white plastic containers (Bartscher) of 14.5 x 11 cm (height x diameter) that were closed on top using a sheet of black plastic mesh (11 x 11 mm) of 11 x 11 cm (length x width) **(Figure S3.4B)**. Containers were strapped together and provided with an identification tag to keep track of individuals. The animals were returned to their housing tank for a 14-days survival period without feeding to emphasise possible vitality differences. Survival assessment was species-specific, based on Kaiser and Spencer (1995), and performed daily on weekdays: common starfish and serpent star were examined for movement of either the body or tube feet; common hermit crab and flying crab were examined for general movements or beating of the maxillipeds; sea mouse was examined for contraction of the longitudinal muscles and curled bodies after light stimulation of their ventral side using a long tie wrap (if necessary, animals were excavated from the sediment first); common whelk was examined for general movement when lightly stimulated on the foot.

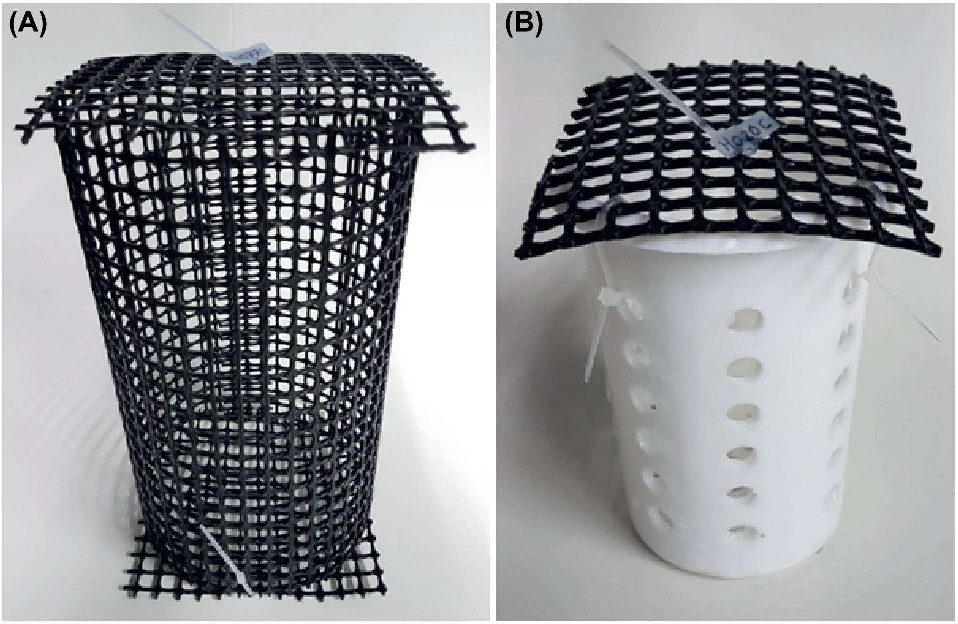

Figure S3.4. Containers used for individual housing during the survival experiment. **(A)** Survival container type used for common starfish, serpent star, sea mouse, and common whelk. **(B)** Survival container type used for common hermit crab and flying crab.

Reference

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Supplementary material 3.5. Statistical methods and output of behavioural measurements.

Statistical data for common starfish (*Asterias rubens*) are given in **Table S3.5A**, **B** & **Figure S3.5A**, **B**, for serpent star (*Ophiura ophiura*) in **Table S3.5C**, **D** & **Figure S3.5C**, **D**, for common whelk (*Buccinum undatum*) in **Table S3.5E**, **F**, **G** & **Figure S3.5E**, **F**, **G**, for sea mouse (*Aphrodita aculeata*) in **Table S3.5H**, **I**, **J** & **Figure S3.5H**, **I**, **J**, for common hermit crab (*Pagurus bernhardus*) in **Table S3.5K**, **L**, **M**, **N** & **Figure S3.5K**, **L**, **M**, **N**, and for flying crab (*Liocarcinus holsatus*) in **Table S3.5O**, **P** & **Figure S3.5O**, **P**.

The effect of treatment on righting duration, walking/crawling duration (not applicable to echinoderms), the duration until the start of burying, and locomotion activity (crabs) was analysed by fitting a linear mixed effect model by Restricted Maximum Likelihood:

$$\mathrm{B}_{i,j} \sim N\big(\alpha + \alpha_i + \beta_1 \mathrm{T}_i + \beta_2 \mathrm{E}_{i,j} + \beta_3 \mathrm{T}_i \times \mathrm{E}_{i,j} + \beta_4 \mathrm{M}_i + \beta_5 \mathrm{L}_i, \sigma^2\big),$$

where $\mathrm{B}_{i,j}$ is the specific behaviour of the *i*th individual (i.e., random effect) at the *j*th event E (i.e., pre- and post-treatment measurement events). α is the overall intercept, α_i the individual-specific intercept, T_i the treatment (i.e., control or exposure), M_i the body mass, and L_i the body length of the *i*th individual and σ represents the residual standard deviation. Because we used a species-specific design, models were applied to each species separately.

Since walking duration for echinoderms and post-treatment righting duration from the moment of emerging with the cephalothorax from the shell for hermit crab were measured once, the potential effects were estimated in linear models (no random effects) using Maximum Likelihood:

$$\mathrm{B}_i \sim N(\alpha + \beta_1 \mathrm{T}_i + \beta_2 \mathrm{M}_i + \beta_3 \mathrm{L}_i, \sigma^2),$$

where B_i is the walking duration of common starfish or serpent star, or the righting duration from the moment of emerging in hermit crab. α is the overall intercept, T_i the treatment (i.e., control or exposure), M_i the body mass, and L_i the body length of the *i*th individual and σ represents the residual standard deviation.

To meet the assumptions of normality and homoscedasticity, all species-specific behaviour data were ln-transformed, except for flying crab, where we applied a power transformation because in four instances a zero value was present in the dataset (animals remained stationary). The power parameter, lambda, was optimised for the area changed and for the number of objects separately (0.265 and 0.357, respectively). Lambda was estimated using the `powerTransform` function within the Yeo-Johnson power family (Yeo and Johnson, 2000) implemented in the `car` package (Fox and Weisberg, 2019). For the fixed effects, we only report the intercept, as well as the fixed effects that were kept constant at the overall mean during the multiple comparison procedure (i.e., body mass and length). Confidence intervals for random effects were calculated using the `confint.merMod` function implemented in the `lme4` package (Bates *et al.*, 2015). The fixed effects of main interest (i.e., treatment, event, and treatment × event) are given as the output of the multiple comparison procedure between the control and exposure group within each event. Significance codes: $p \leq 0.001$ ***, ≤ 0.01 **, ≤ 0.05 *, > 0.05 n.s.

To test whether sample sizes were sufficient to detect relevant effects, given the standard deviations (SD) and for a power of 85%, we performed a power analysis based on a t-test with the `pwr.t2n.test` function in the `pwr` package (Champely, 2020). Subsequently, we calculated Cohen's *d* (Cohen, 1988) and the corresponding mean of the exposure group, as an estimate of the effect size we could have detected. Power analyses were performed for all models where we did not find a significant effect of treatment or where we did not perform a sensitivity analysis. Outcomes are provided in the caption of the table with the respective model output. This simplified power analysis does not take multiple fixed effects, interactions, and random effects into account, and may thus underestimate the power of the models depending on the effect sizes of these parameters. Results show that current sample sizes provide ample power to detect significant differences that would be interesting (i.e., indicate a negative effect of the treatment).

Table S3.5A. Fixed effects, random effects, and multiple comparisons for ln-transformed righting duration in starfish, estimated in a linear mixed effect model by Restricted Maximum Likelihood. No significant effect of treatment was found. Measured righting durations are shown in **Figure 3.2A** of the manuscript. If the mean of the exposure group would have been 0.90 SD larger in the sixth righting event (i.e., 2.99 min instead of 2.14 min), we would have a power of 85% with current sample size.

Fixed effects						
	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	0.9980	0.2699	89.2895	3.698	0.0004	***
body mass	0.0086	0.0040	81.1214	2.123	0.0368	*
body length	−0.0578	0.0558	80.9966	−1.037	0.3030	n.s.

Random effects			
	Variance	**2.5 % confidence level**	**97.5 % confidence level**
individual identity	0.0946	0.0632	0.1293
residual	0.1699	0.1503	0.1837

Multiple comparisons								
Timing	**Righting event**	**Number of animals**		**Estimate**	**Standard error**	***z*-score**	***p*-value**	**Sig.**
		Control	**Exposure**					
Pre-treatment	1	44	41	−0.0436	0.1122	−0.388	1.000	n.s.
	2	44	41	−0.0476	0.1122	−0.424	1.000	n.s.
	3	44	41	0.1824	0.1122	1.625	0.572	n.s.
	4	44	41	0.1783	0.1122	1.589	0.600	n.s.
	5	44	41	0.2245	0.1122	2.001	0.308	n.s.
Post-treatment	6	44	41	0.1019	0.1122	0.908	0.976	n.s.
	7	44	41	0.1485	0.1122	1.323	0.799	n.s.
	8	44	41	0.1562	0.1122	1.392	0.751	n.s.
	9	43[a]	41	−0.0413	0.1127	−0.366	1.000	n.s.
	10	43[a]	41	0.1212	0.1127	1.076	0.930	n.s.

[a] righting duration from one starfish specimen in the 9th and 10th righting event was missing (out of 850 measurements) due to failure of camera equipment.

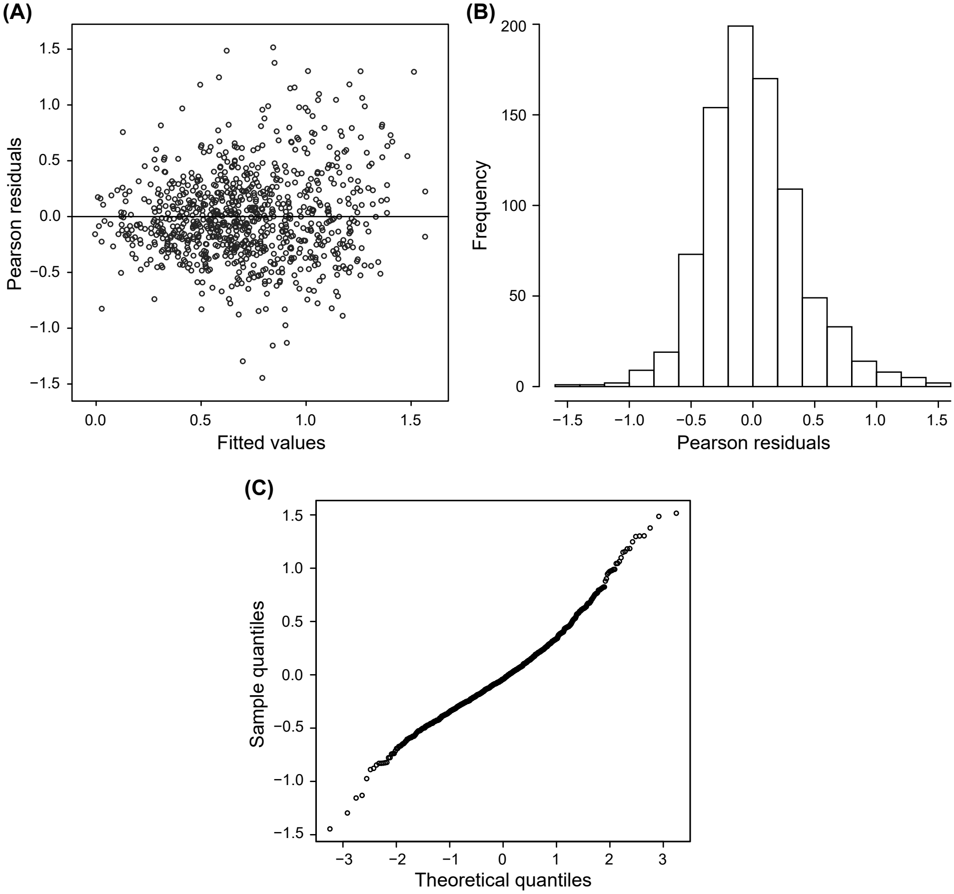

Figure S3.5A. Regression diagnostics of the starfish righting duration model as provided in **Table S3.5A**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5B. Ln-transformed walking duration of starfish as function of the exposure and control treatment (i.e., exposed to the electrical pulse stimulus or not) estimated in a linear model by Maximum Likelihood with body mass and length as additional fixed effects. No significant effect of treatment was found. Measured walking durations are shown in **Figure 3.2C** of the manuscript. If the mean of the exposure group would have been 0.47 SD larger (i.e., 1.45 min instead of 1.06 min), we would have a power of 85% with current sample size.

	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	1.0340	0.5424	78	1.906	0.0603	n.s.
exposure	0.0338	0.1450	78	0.233	0.8161	n.s.
body mass	−0.0004	0.0078	78	−0.045	0.9644	n.s.
body length	−0.1908	0.1124	78	−1.697	0.0936	n.s.

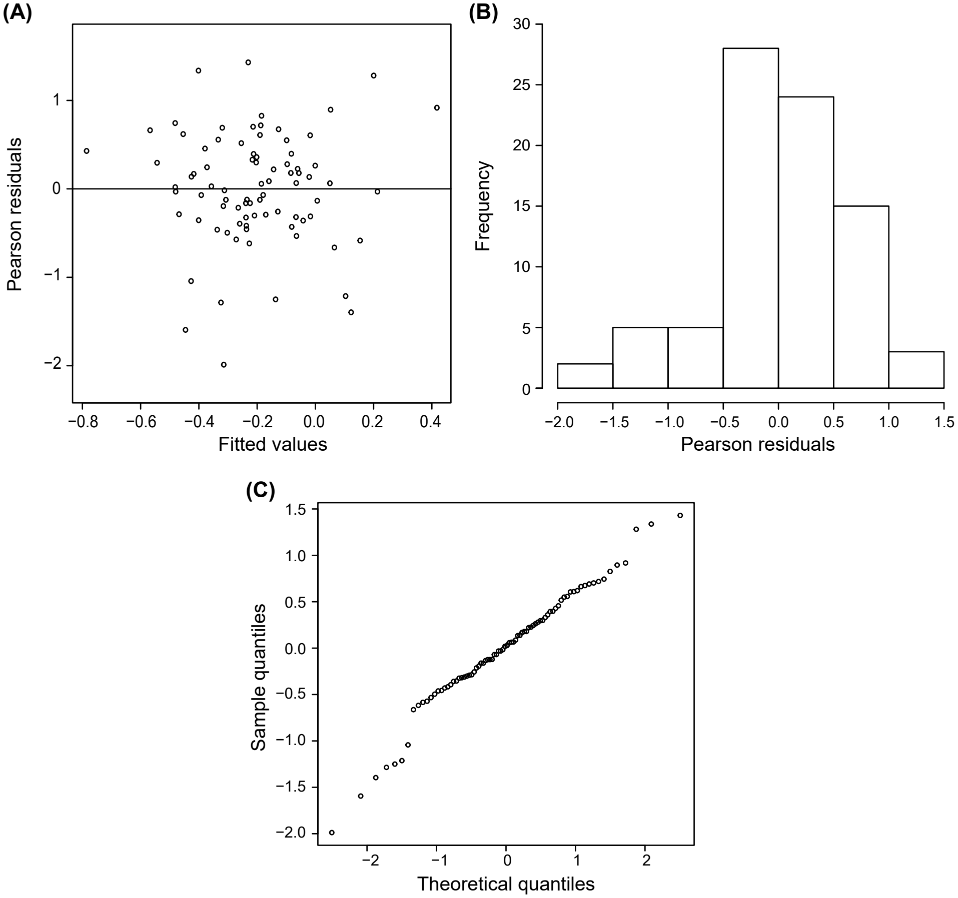

Figure S3.5B. Regression diagnostics of the starfish walking duration model as provided in **Table S3.5B**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5C. Fixed effects, random effects, and multiple comparisons for ln-transformed righting duration in serpent star, estimated in a linear mixed effect model by Restricted Maximum Likelihood. No significant effect of treatment was found. Measured righting durations are shown in **Figure 3.2B** of the manuscript. If the mean of the exposure group would have been 1.07 SD larger in the sixth righting event (i.e., 2.7 s instead of 2.0 s), we would have a power of 85% with current sample size.

Fixed effects

	Estimate	Standard error	*df*	*t*-value	*p*-value	Sig.
intercept	1.5502	0.4563	39.0967	3.397	0.0016	**
body mass	0.2236	0.1286	37.9905	1.738	0.0903	n.s.
body length	−0.8275	0.4189	37.9815	−1.976	0.0555	n.s.

Random effects

	Variance	2.5 % confidence level	97.5 % confidence level
individual identity	0.0148	0.0064	0.0246
residual	0.0705	0.0584	0.0777

Multiple comparisons

Timing	Righting event	Number of animals		Estimate	Standard error	*z*-score	*p*-value	Sig.
		Control	Exposure					
Pre-treatment	1	21	21	0.1148	0.0942	1.219	0.894	n.s.
	2	21	21	−0.0584	0.0942	−0.620	0.999	n.s.
	3	21	21	−0.0689	0.0942	−0.732	0.997	n.s.
	4	21	21	0.2141	0.0942	2.273	0.191	n.s.
	5	21	21	0.1121	0.0942	1.190	0.907	n.s.
Post-treatment	6	21	21	0.0482	0.0942	0.512	1.000	n.s.
	7	21	21	0.1427	0.0942	1.515	0.706	n.s.
	8	21	21	0.0042	0.0942	0.044	1.000	n.s.
	9	21	21	−0.0600	0.0942	−0.637	0.999	n.s.
	10	21	20[a]	−0.0015	0.0950	−0.015	1.000	n.s.

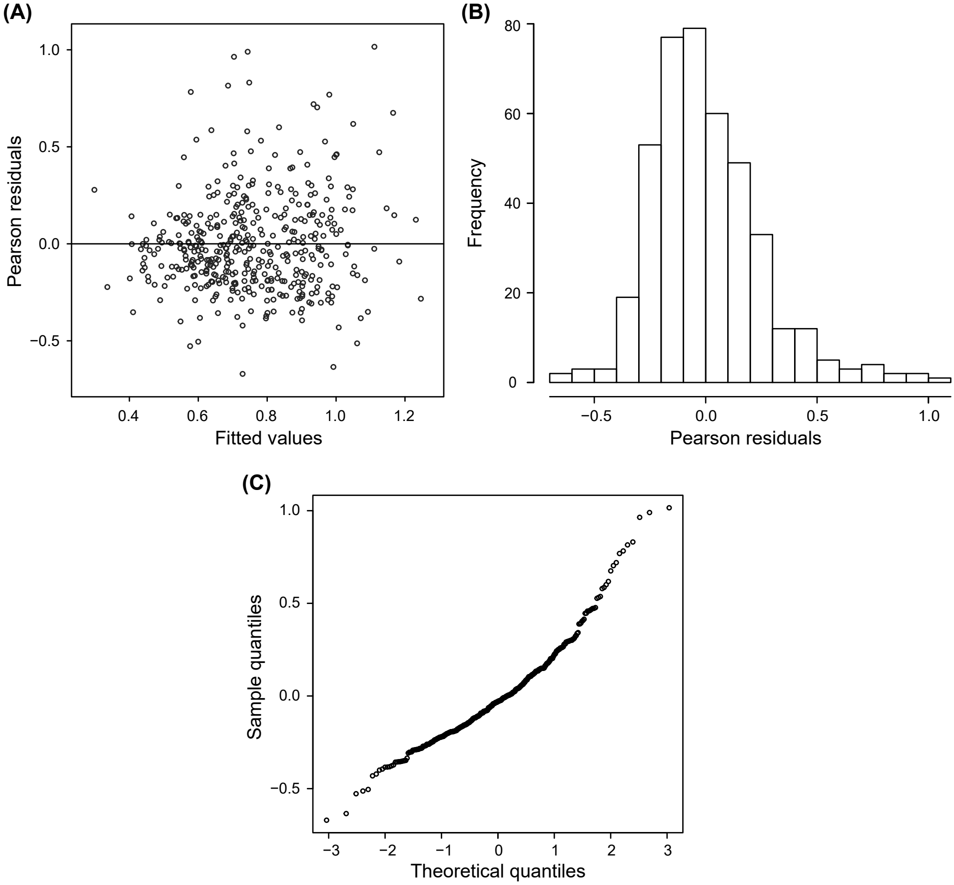

Figure S3.5C. Regression diagnostics of the serpent star righting duration model as provided in **Table S3.5C**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5D. Ln-transformed walking duration of serpent star as function of the exposure and control treatment (i.e., exposed to the electrical pulse stimulus or not) estimated in a linear model by Maximum Likelihood with body mass and length as additional fixed effects. No significant effect of treatment was found. Measured walking durations are shown in **Figure 3.2D** of the manuscript. If the mean of the exposure group would have been 1.25 SD larger (i.e., 10.3 s instead of 8.0 s), we would have a power of 85% with current sample size.

	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	4.9516	2.0513	20	2.414	0.0255	*
exposure	0.1988	0.1431	20	1.389	0.1801	n.s.
body mass	0.4165	0.3947	20	1.055	0.3039	n.s.
body length	–2.2534	1.6481	20	–1.367	0.1867	n.s.

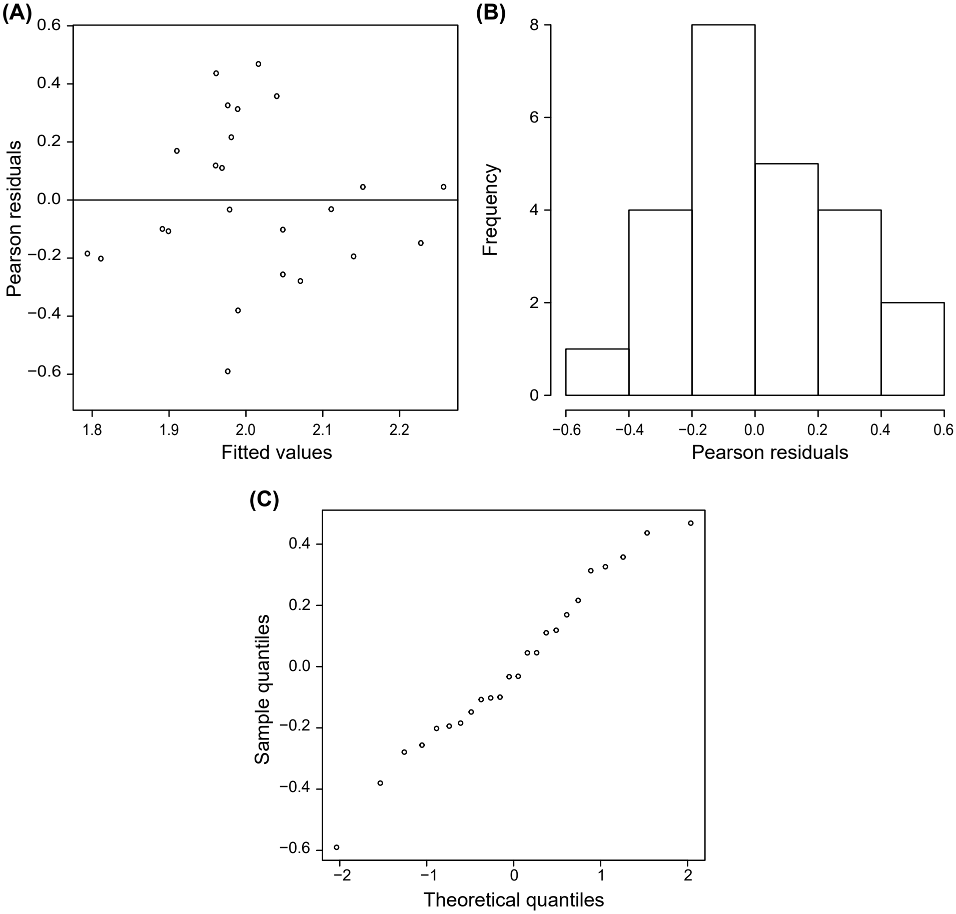

Figure S3.5D. Regression diagnostics of the serpent star walking duration model as provided in **Table S3.5D**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5E. Fixed effects, random effects, and multiple comparisons for ln-transformed righting duration in whelk, estimated in a linear mixed effect model by Restricted Maximum Likelihood. No significant effect of treatment was found. Measured righting durations are shown in **Figure 3.3A** of the manuscript. If the mean of the exposure group would have been 0.59 SD larger in the second righting event (i.e., 4.15 min instead of 2.74 min), we would have a power of 85% with current sample size.

Fixed effects						
	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	0.4564	0.4869	84.39	0.937	0.3513	n.s.
body mass	0.0003	0.0058	83.00	0.054	0.9569	n.s.
body length	0.1563	0.1104	83.00	1.416	0.1606	n.s.

Random effects			
	Variance	**2.5 % confidence level**	**97.5 % confidence level**
individual identity	0.1471	0.0738	0.2246
residual	0.1816	0.1337	0.2426

Multiple comparisons								
Timing	**Righting event**	**Number of animals**		**Estimate**	**Standard error**	***z*-score**	***p*-value**	**Sig.**
		Control	**Exposure**					
Pre-treatment	1	46	41	−0.2069	0.1233	−1.678	0.167	n.s.
Post-treatment	2	46	41	−0.1199	0.1233	−0.973	0.527	n.s.

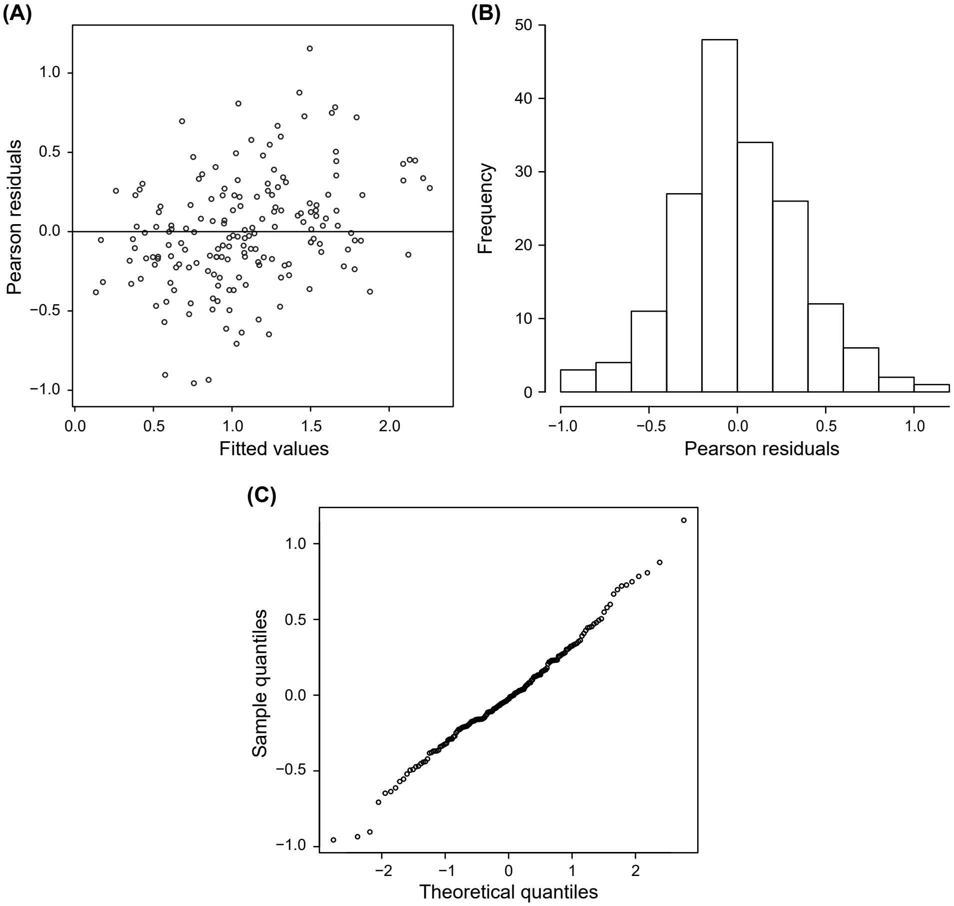

Figure S3.5E. Regression diagnostics of the whelk righting duration model as provided in **Table S3.5E**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5F. Fixed effects, random effects, and multiple comparisons for ln-transformed duration until the start of burying in whelk, estimated in a linear mixed effect model by Restricted Maximum Likelihood. No significant effect of treatment was found. Measured durations until the start of burying are shown in **Figure 3.3B** of the manuscript. If the mean of the exposure group would have been 1.46 SD larger in the second burying event (i.e., 1.39 min instead of 0.49 min), we would have a power of 85% with current sample size.

Fixed effects

	Estimate	Standard error	*df*	*t*-value	*p*-value	Sig.
intercept	–3.1927	1.408	48.9188	–2.267	0.0279	*
body mass	–0.0338	0.0233	50.6385	–1.452	0.1528	n.s.
body length	0.5728	0.3560	48.7948	1.609	0.1141	n.s.

Random effects

	Variance	2.5 % confidence level	97.5 % confidence level
individual identity	0.5277	0.1480	0.9219
residual	0.6186	0.3863	0.9545

Multiple comparisons

Timing	Burying event	Number of animals		Estimate	Standard error	*z*-score	*p*-value	Sig.
		Control	Exposure					
Pre-treatment	1	25	21	0.2511	0.3145	0.798	0.651	n.s.
Post-treatment	2	26	17	–0.5583	0.3283	–1.701	0.162	n.s.

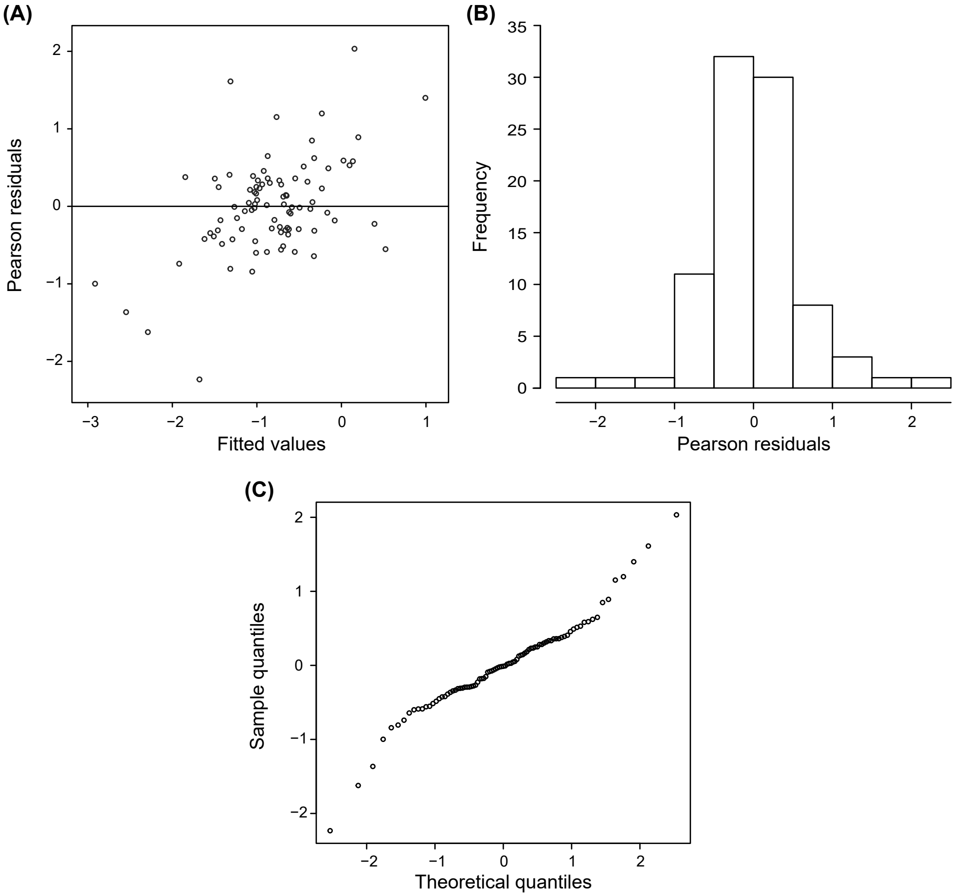

Figure S3.5F. Regression diagnostics of the whelk duration until the start of burying model as provided in **Table S3.5F**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5G. Fixed effects, random effects, and multiple comparisons for ln-transformed crawling duration in whelk, estimated in a linear mixed effect model by Restricted Maximum Likelihood. No significant effect of treatment was found. Measured crawling durations are shown in **Figure 3.3C** of the manuscript. If the mean of the exposure group would have been 1.60 SD larger in the second crawling event (i.e., 5.29 min instead of 2.74 min), we would have a power of 85% with current sample size.

Fixed effects

	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	1.5200	0.7032	40.0584	2.161	0.0367	*
body mass	0.0102	0.0066	35.2476	1.555	0.1290	n.s.
body length	−0.1235	0.1444	37.9440	−0.855	0.3977	n.s.

Random effects

	Variance	**2.5 % confidence level**	**97.5 % confidence level**
individual identity	0.1073	0.0000	0.2127
residual	0.1636	0.0956	0.2752

Multiple comparisons

Timing	**Crawling event**	**Number of animals**		**Estimate**	**Standard error**	***z*-score**	***p*-value**	**Sig.**
		Control	**Exposure**					
Pre-treatment	1	20	18	−0.2052	0.1679	−1.222	0.382	n.s.
Post-treatment	2	18	21	−0.2792	0.1657	−1.685	0.170	n.s.

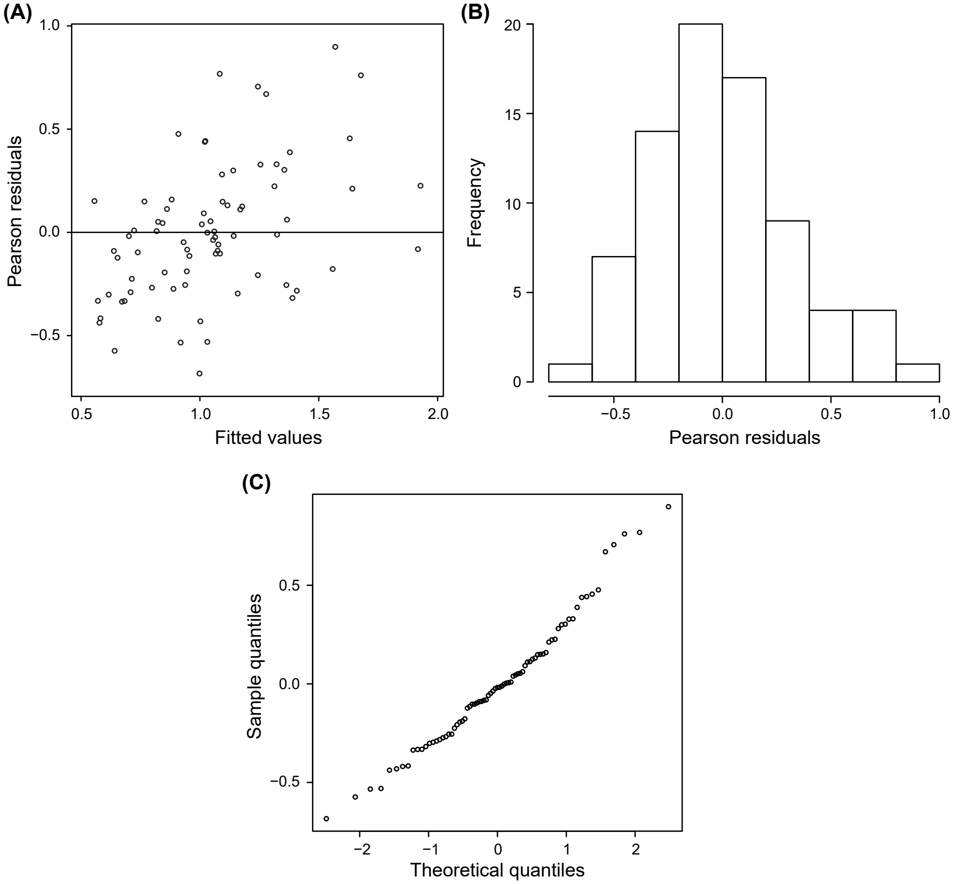

Figure S3.5G. Regression diagnostics of the whelk crawling duration model as provided in **Table S3.5G**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5H. Fixed effects, random effects, and multiple comparisons for ln-transformed righting duration in sea mouse, estimated in a linear mixed effect model by Restricted Maximum Likelihood. For 5 control and 8 exposed sea mouse, post-treatment righting duration was longer than 10 min, leading to missing values. To check whether these missing data may have biased our results, we performed a sensitivity analysis in which we included the specimens by assigning a value of 11 min to the 5 control animals and a value of 100 min (10 times the time limit) for the 8 exposed animals. No significant effect of treatment was found. Measured righting durations are shown in **Figure 3.4A** of the manuscript.

Fixed effects						
	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	3.2029	1.1194	83.2302	2.861	0.0053	**
body mass	0.1528	0.0596	82.0000	2.548	0.0127	*
body length	–0.7841	0.2605	82.0000	–3.011	0.0035	**

Random effects			
	Variance	**2.5 % confidence level**	**97.5 % confidence level**
individual identity	0.6024	0.2850	0.9366
residual	0.8210	0.6032	1.0984

Multiple comparisons								
Timing	**Righting event**	**Number of animals**		**Estimate**	**Standard error**	***z*-score**	***p*-value**	**Sig.**
		Control	**Exposure**					
Pre-treatment	1	44	42	0.0790	0.2600	0.304	0.9371	n.s.
Post-treatment	2	44[a]	42[b]	0.5542	0.2600	2.132	0.0619	n.s.

[a] 5 missing values in the control group were given a value of 11 min; [b] 8 missing values in the control group were given a value of 100 minutes.

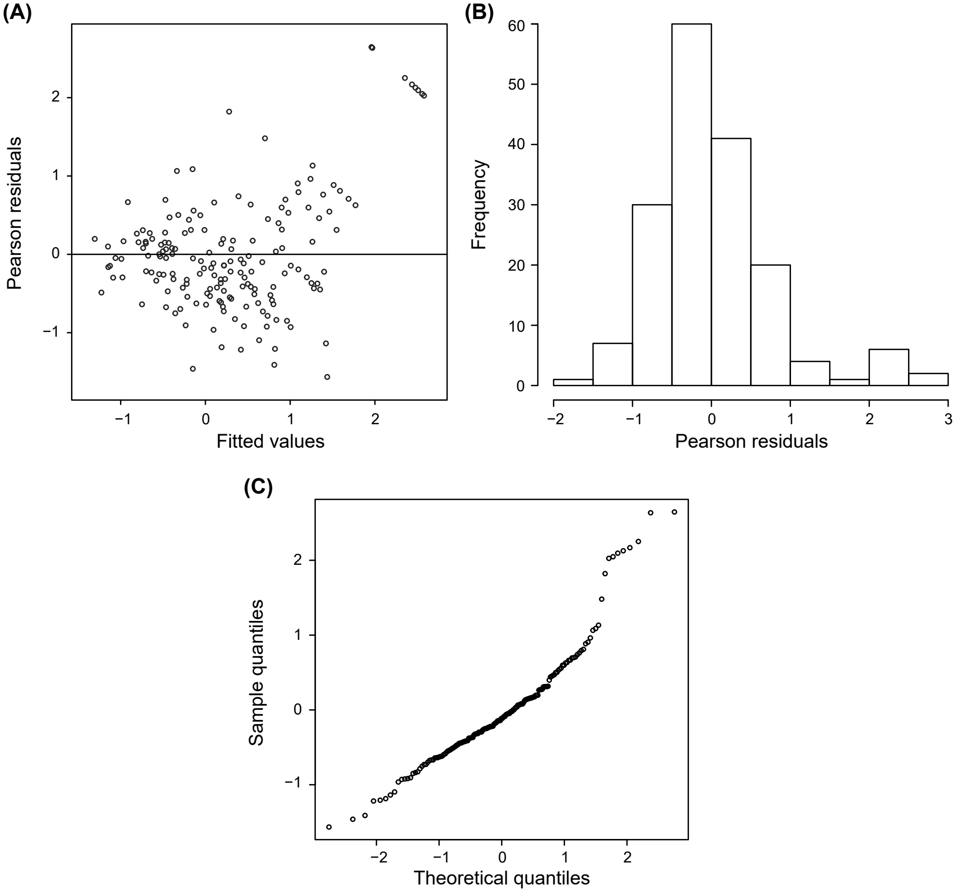

Figure S3.5H. Regression diagnostics of the sea mouse righting duration model as provided in **Table S3.5H**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot. Please note that for 5 control and 8 exposed sea mouse, post-treatment righting durations were longer than 10 min, leading to missing values. To check whether these missing data may have biased our results, we performed a sensitivity analysis in which we included the specimens by assigning a value of 11 min to the 5 control animals and a value of 100 min (10 times the time limit) for the 8 exposed animals. The extreme values in this figure correspond to these 8 exposure group animals.

Table S3.5I. Fixed effects, random effects, and multiple comparisons for ln-transformed duration until the start of burying in sea mouse, estimated in a linear mixed effect model by Restricted Maximum Likelihood. No significant effect of treatment was found. Measured durations until the start of burying are shown in **Figure 3.4B** of the manuscript. If mean of the exposure group would have been 0.29 SD larger in the second burying event (i.e., 41.9 s instead of 32.2 s), we would have a power of 85% with current sample size.

Fixed effects

	Estimate	Standard error	*df*	*t*-value	*p*-value	Sig.
intercept	−1.0212	0.8199	46.4428	−1.246	0.2192	n.s.
body mass	0.0282	0.0430	46.0770	0.657	0.5143	n.s.
body length	−0.1355	0.1918	46.0645	−0.706	0.4837	n.s.

Random effects

	Variance	2.5 % confidence level	97.5 % confidence level
individual identity	0.1911	0.0083	0.3635
residual	0.3324	0.2171	0.5120

Multiple comparisons

Timing	Burying event	Number of animals		Estimate	Standard error	*z*-score	*p*-value	Sig.
		Control	Exposure					
Pre-treatment	1	30	23	0.2528	0.1993	1.268	0.355	n.s.
Post-treatment	2	35	25	0.2253	0.1907	1.182	0.405	n.s.

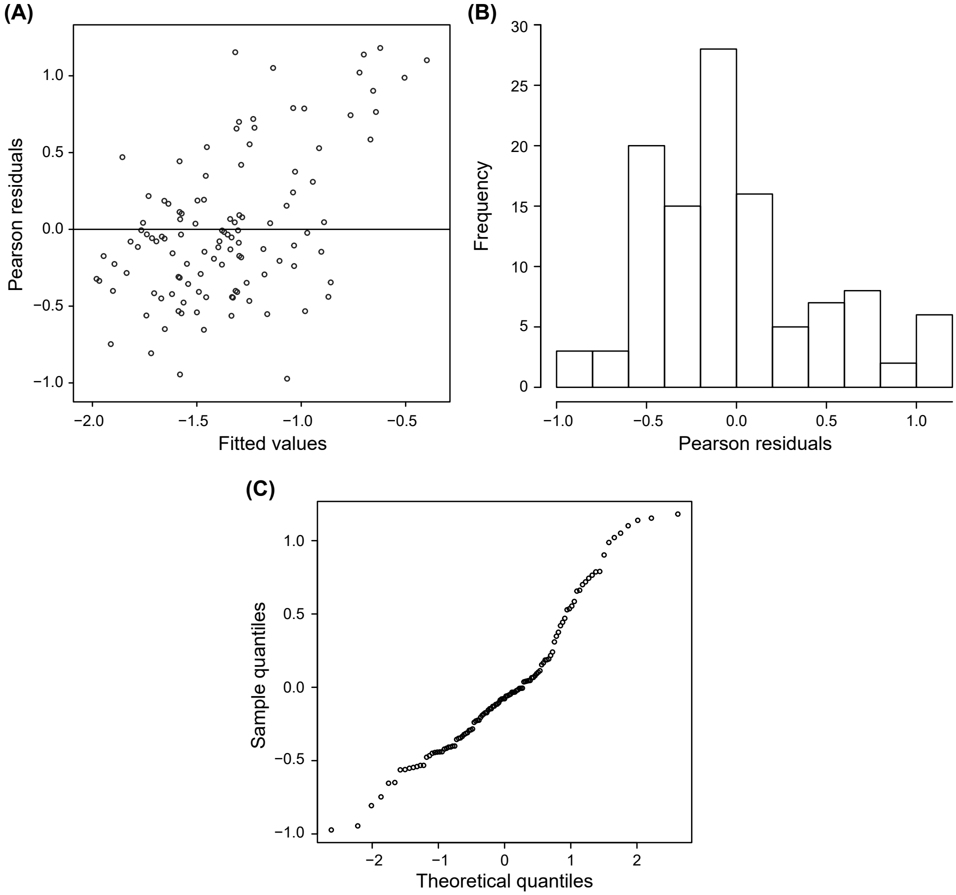

Figure S3.5I. Regression diagnostics of the sea mouse duration until the start of burying model as provided in **Table S3.5I**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5J. Fixed effects, random effects, and multiple comparisons for ln-transformed walking duration in sea mouse, estimated in a linear mixed effect model by Restricted Maximum Likelihood. No significant effect of treatment was found. Measured crawling durations are shown in **Figure 3.4C** of the manuscript. If the mean of the exposure group would have been 0.75 SD larger in the second walking event (i.e., 108.0 s instead of 73.0 s), we would have a power of 85% with current sample size.

Fixed effects

	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	1.0317	0.8080	28.3577	1.277	0.212	n.s.
body mass	–0.0134	0.0441	27.7311	–0.305	0.763	n.s.
body length	–0.1735	0.1852	28.0326	–0.937	0.357	n.s.

Random effects

	Variance	**2.5 % confidence level**	**97.5 % confidence level**
individual identity	0.1135	0.0141	0.2132
residual	0.1213	0.0672	0.2098

Multiple comparisons

Timing	**Walking event**	**Number of animals**		**Estimate**	**Standard error**	***z*-score**	***p*-value**	**Sig.**
		Control	**Exposure**					
Pre-treatment	1	14	19	0.1812	0.1821	0.995	0.509	n.s.
Post-treatment	2	9	17	0.2097	0.2016	1.040	0.480	n.s.

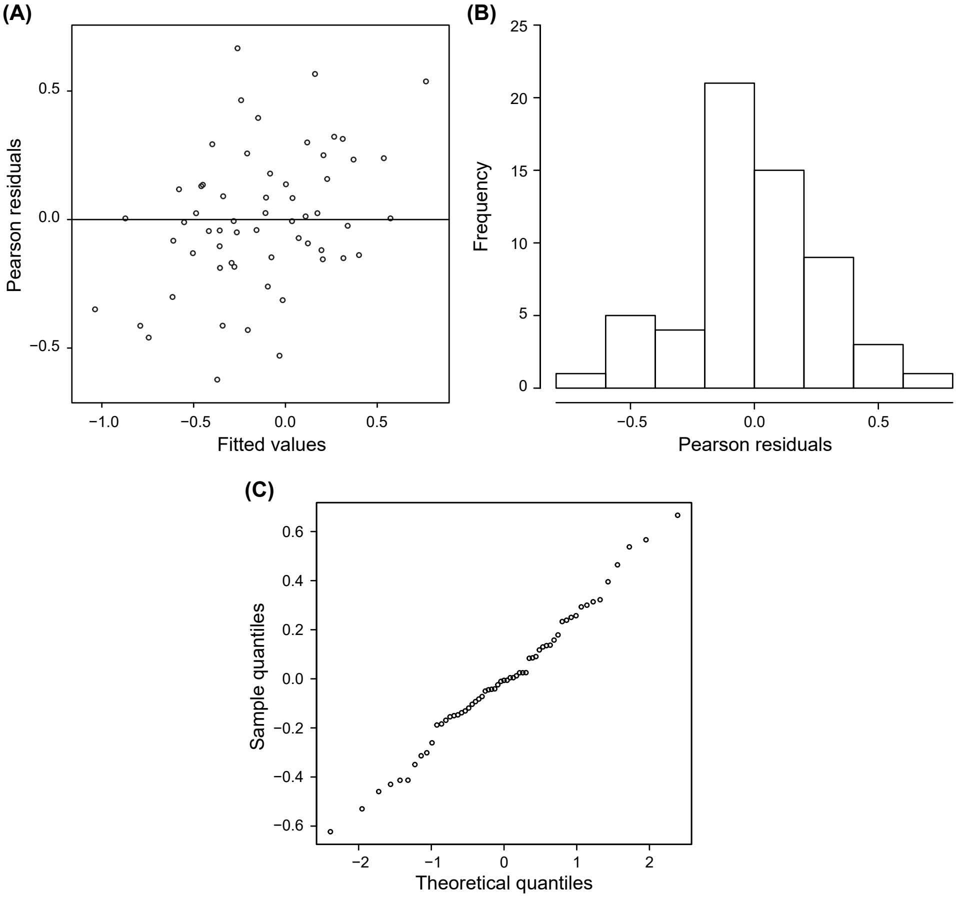

Figure S3.5J. Regression diagnostics of the sea mouse walking duration model as provided in **Table S3.5J**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5K. Fixed effects, random effects, and multiple comparisons for ln-transformed righting duration in hermit crab, estimated in a linear mixed effect model by Restricted Maximum Likelihood. Post-treatment righting duration of the exposure group was significantly longer than in the control group. Measured righting durations are shown in **Figure 3.5A** of the manuscript.

Fixed effects

	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	−3.0620	0.6819	83.8298	−4.491	<0.0001	***
body mass	−0.0094	0.0350	82.4803	−0.269	0.7887	n.s.
body length	0.2492	0.2590	82.4471	0.962	0.3388	n.s.

Random effects

	Variance	**2.5 % confidence level**	**97.5 % confidence level**
individual identity	1.1986	0.8039	1.6307
residual	0.3729	0.2733	0.5000

Multiple comparisons

Timing	**Righting event**	**Number of animals**		**Estimate**	**Standard error**	***z*-score**	***p*-value**	**Sig.**
		Control	**Exposure**					
Pre-treatment	1	43	43	0.0755	0.2725	0.277	0.9286	n.s.
Post-treatment	2	43	42[a]	1.0394	0.2730	3.807	0.0003	***

[a] righting duration from one hermit crab specimen in the 2nd righting event was missing (out of 172 measurements) due to failure of camera equipment.

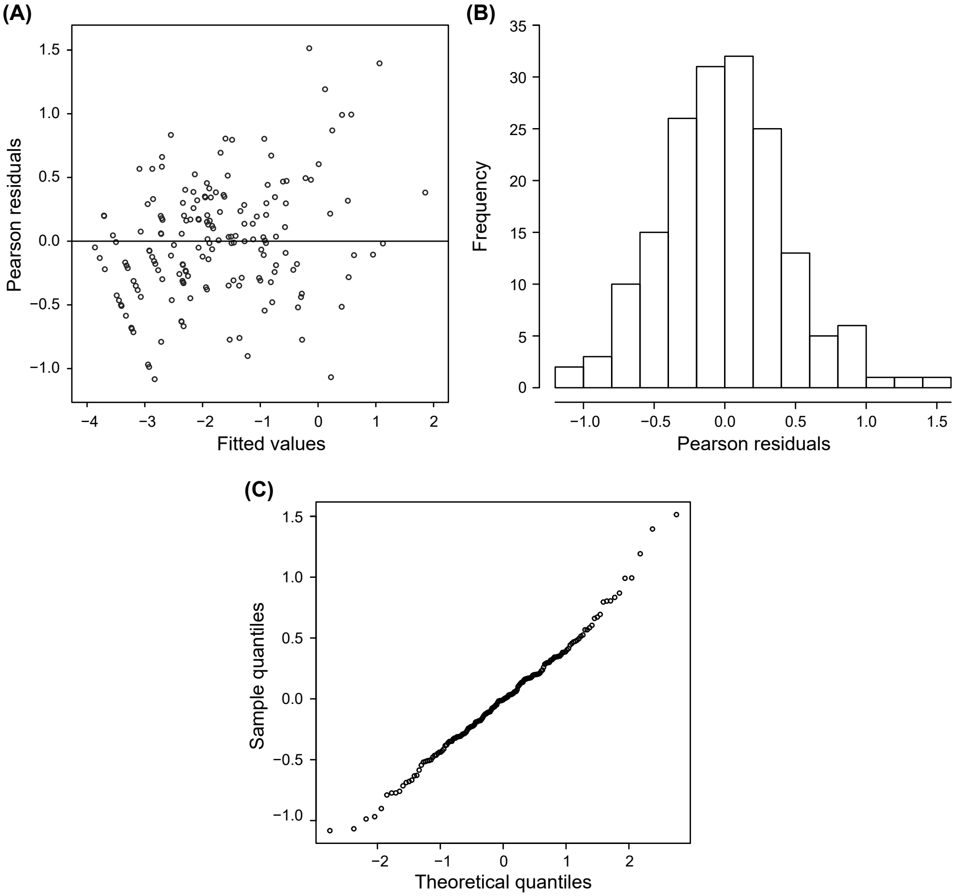

Figure S3.5K. Regression diagnostics of the hermit crab righting duration model as provided in **Table S3.5K**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5L. Ln-transformed post-treatment righting duration measured from the moment of emerging with the cephalothorax from the shell in hermit crab as function of the exposure and control treatment (i.e., exposed to the electrical pulse stimulus or not) estimated in a linear model by Maximum Likelihood with body mass and length as additional fixed effects. No significant effect of treatment was found. Measured righting durations from the moment of emerging are shown in **Figure 3.5B** of the manuscript. If the mean of the exposure group would have been 0.50 SD larger (i.e., 14.9 s instead of 9.1 s), we would have a power of 85% with current sample size.

	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	0.6234	0.6188	81	1.007	0.317	n.s.
exposure	0.3817	0.2295	81	1.663	0.100	n.s.
body mass	–0.0225	0.0321	81	–0.702	0.485	n.s.
body length	0.2214	0.2372	81	0.933	0.353	n.s.

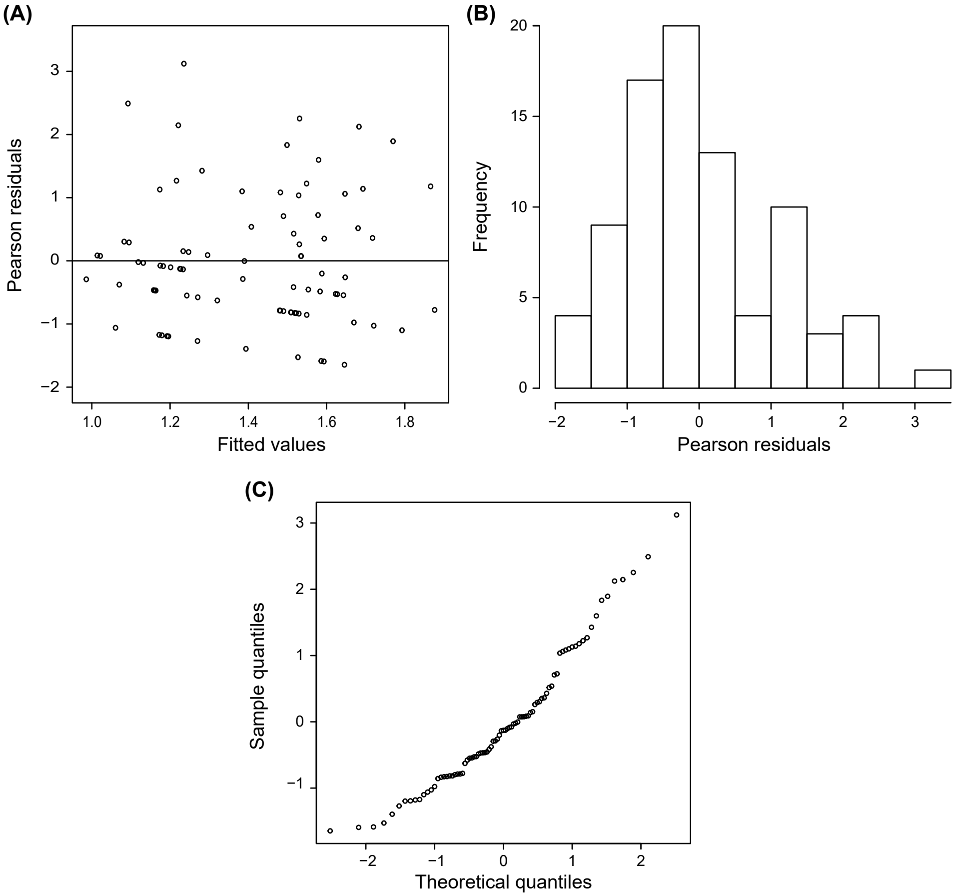

Figure S3.5L. Regression diagnostics of the hermit crab post-treatment righting duration measured from the moment of emerging with the cephalothorax from the shell model as provided in **Table S3.5L**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5M. Fixed effects, random effects, and multiple comparisons for ln-transformed area changed as proxy for whole-body movements in hermit crab, estimated in a linear mixed effect model by Restricted Maximum Likelihood. Post-treatment whole-body movement of the exposure group was significantly lower than in the control group. Measured whole-body movements are shown in **Figure 3.5C** of the manuscript.

Fixed effects						
	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	14.3975	0.5932	84.5212	24.271	<0.0001	***
body mass	0.0470	0.0304	82.0000	1.546	0.1259	n.s.
body length	0.2104	0.2246	82.0000	0.937	0.3515	n.s.

Random effects			
	Variance	**2.5 % confidence level**	**97.5 % confidence level**
individual identity	0.8186	0.5155	1.1491
residual	0.4582	0.3367	0.6130

Multiple comparisons								
Timing	**Locomotion event**	**Number of animals**		**Estimate**	**Standard error**	***z*-score**	***p*-value**	**Sig.**
		Control	**Exposure**					
Pre-treatment	1	43	43	−0.1764	0.2455	−0.719	0.6693	n.s.
Post-treatment	2	43	43	−0.5451	0.2455	−2.220	0.0465	*

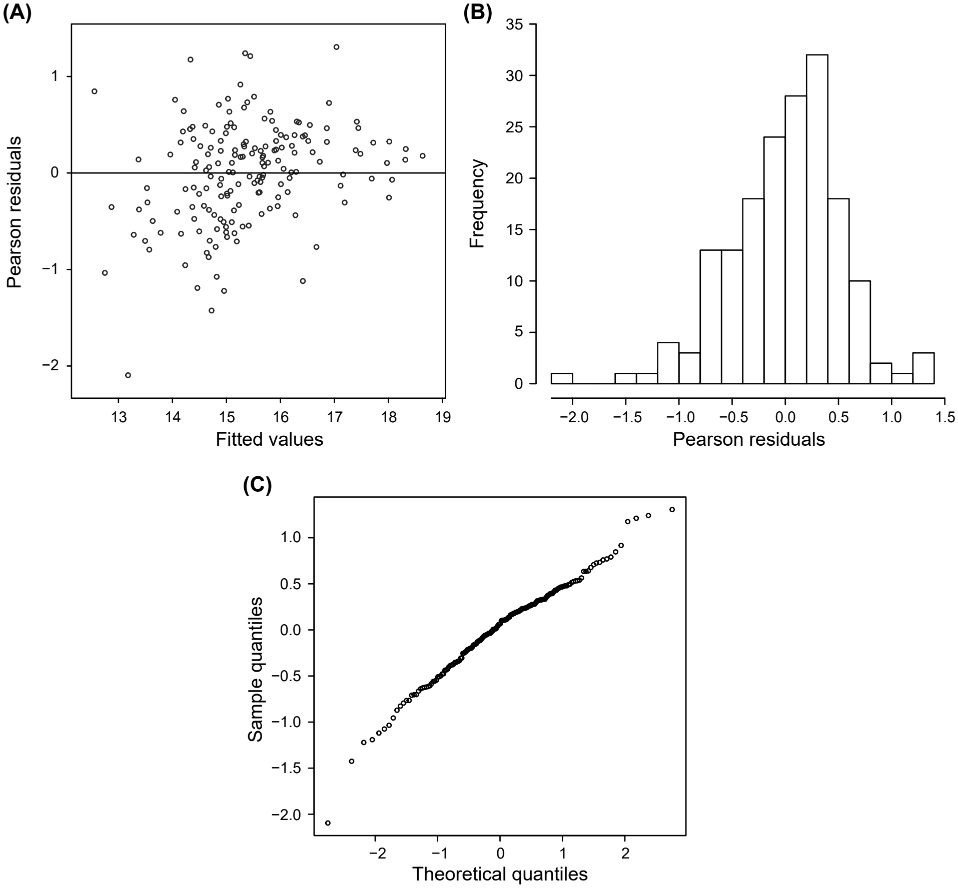

Figure S3.5M. Regression diagnostics of the hermit crab area changed as proxy for whole-body movements model as provided in **Table S3.5M**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5N. Fixed effects, random effects, and multiple comparisons for ln-transformed number of moving objects as proxy for body extremity movements in hermit crab, estimated in a linear mixed effect model by Restricted Maximum Likelihood. No significant effect of treatment was found. Measured body extremity movements are shown in **Figure 3.5D** of the manuscript. If the mean of the exposure group would have been 0.90 SD larger in the second locomotion event, we would have a power of 85% with current sample size.

Fixed effects						
	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	9.0585	0.4792	84.4943	18.903	<0.0001	***
body mass	0.0273	0.0245	82.0000	1.112	0.2696	n.s.
body length	0.1434	0.1814	82.0000	0.790	0.4316	n.s.

Random effects			
	Variance	**2.5 % confidence level**	**97.5 % confidence level**
individual identity	0.5359	0.3382	0.7515
residual	0.2959	0.2174	0.3958

Multiple comparisons								
Timing	**Locomotion event**	**Number of animals**		**Estimate**	**Standard error**	***z*-score**	***p*-value**	**Sig.**
		Control	**Exposure**					
Pre-treatment	1	43	43	−0.1146	0.1982	−0.579	0.766	n.s.
Post-treatment	2	43	43	−0.2938	0.1982	−1.483	0.223	n.s.

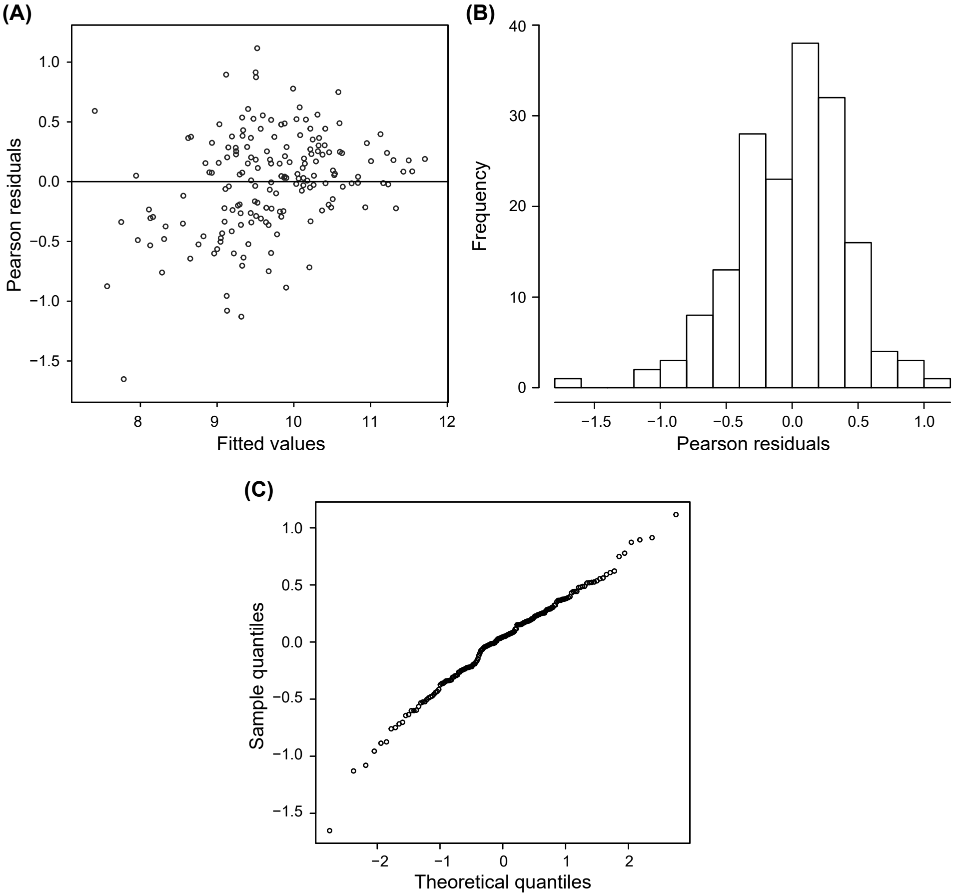

Figure S3.5N. Regression diagnostics of the hermit crab number of moving objects as proxy for body extremity movements model as provided in **Table S3.5N**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5O. Fixed effects, random effects, and multiple comparisons for power-transformed area changed as proxy for whole-body movements in flying crab, estimated in a linear mixed effect model by Restricted Maximum Likelihood. Post-treatment whole-body movement of the exposure group was significantly lower than in the control group. Measured whole-body movements are shown in **Figure 3.6A** of the manuscript.

Fixed effects						
	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	58.573	44.111	86.291	1.328	0.1877	n.s.
body mass	2.961	1.744	86.000	1.697	0.0933	n.s.
body length	–9.611	18.132	86.000	–0.530	0.5974	n.s.

Random effects			
	Variance	**2.5 % confidence level**	**97.5 % confidence level**
individual identity	140.9	45.4334	238.2256
residual	302.2	223.6513	401.7453

Multiple comparisons								
Timing	**Locomotion event**	**Number of animals**		**Estimate**	**Standard error**	***z*-score**	***p*-value**	**Sig.**
		Control	**Exposure**					
Pre-treatment	1	46	44	4.722	4.451	1.061	0.4818	n.s.
Post-treatment	2	46	44	–10.473	4.451	–2.353	0.0362	*

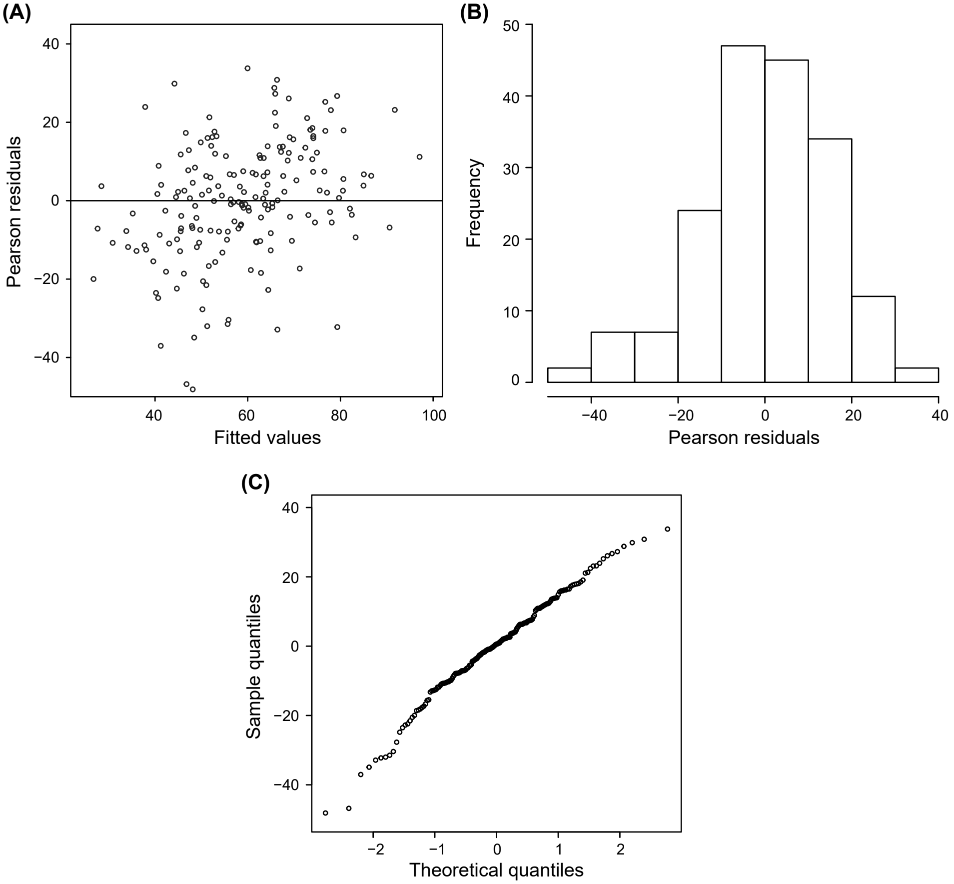

Figure S3.5O. Regression diagnostics of the flying crab area changed as proxy for whole-body movements model as provided in **Table S3.5O**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of Pearson residuals, and **(C)** normal quantile-quantile scatter plot.

Table S3.5P. Fixed effects, random effects, and multiple comparisons for power-transformed number of objects as proxy for body extremity movements in flying crab, estimated in a linear mixed effect model by Restricted Maximum Likelihood. Post-treatment body extremity movement of the exposure group was significantly lower than in the control group. Measured body extremity movements are shown in **Figure 3.6B** of the manuscript.

Fixed effects						
	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	24.8938	23.7632	86.2830	1.048	0.2978	n.s.
body mass	1.4964	0.9398	86.0000	1.592	0.1150	n.s.
body length	−3.5108	9.7680	86.0000	−0.359	0.7202	n.s.

Random effects			
	Variance	**2.5 % confidence level**	**97.5 % confidence level**
individual identity	42.07	14.6025	70.2012
residual	85.35	63.1576	113.4504

Multiple comparisons								
	Locomotion	**Number of animals**			**Standard**			
Timing	**event**	**Control**	**Exposure**	**Estimate**	**error**	***z*-score**	***p*-value**	**Sig.**
Pre-treatment	1	46	44	1.624	2.387	0.680	0.7352	n.s.
Post-treatment	2	46	44	−5.774	2.387	−2.419	0.0302	*

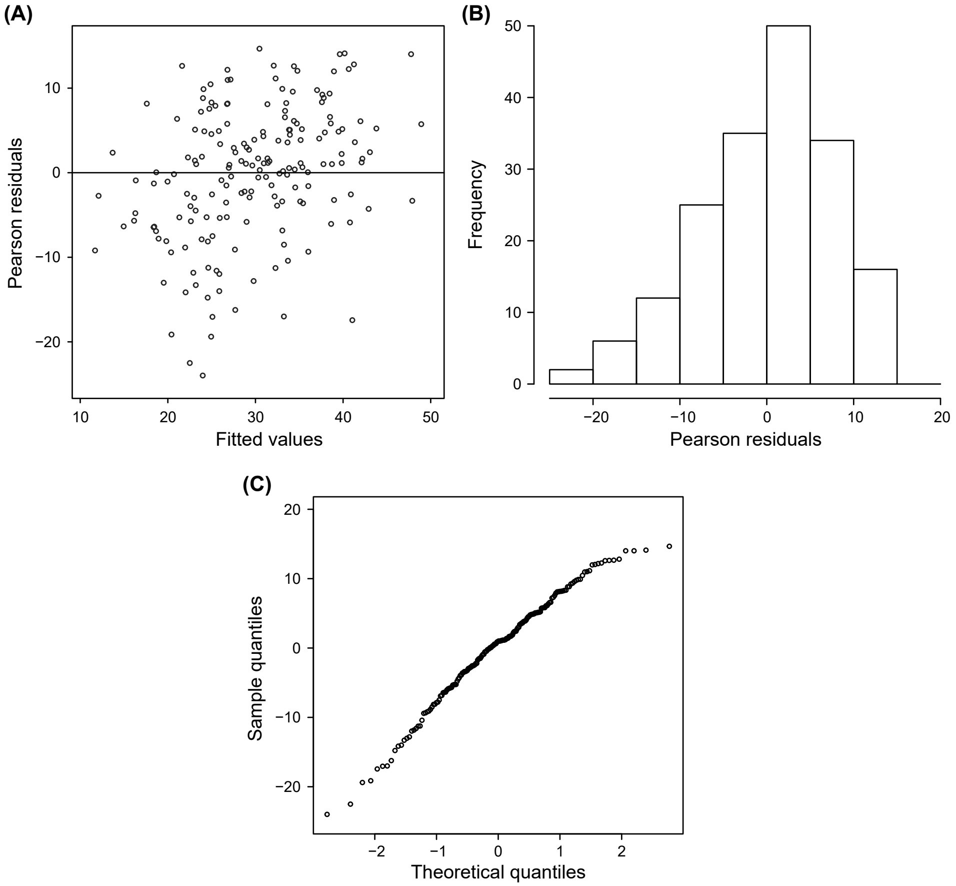

Figure S3.5P. Regression diagnostics of the flying crab number of moving objects as proxy for body extremity movements model as provided in **Table S3.5P**. **(A)** Scatter plot of Pearson residuals versus fitted values, **(B)** histogram of residuals, and **(C)** normal quantile-quantile scatter plot.

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Yeo, I.-K., and Johnson, R. A. 2000. A new family of power transformations to improve normality or symmetry. Biometrika, 87: 954–959

Supplementary material 3.6. Statistical methods and output of survival measurements.

Supporting information on the statistical analyses of the survival measurements for common starfish (*Asterias rubens*), serpent star (*Ophiura ophiura*), common whelk (*Buccinum undatum*), sea mouse (*Aphrodita aculeata*), common hermit crab (*Pagurus bernhardus*), and flying crab (*Liocarcinus holsatus*) (**Table S3.6A**, **B**). The effect of electrical exposure on survival at 14 days after the behaviour measurements was assessed by fitting a generalized linear model by Maximum Likelihood and a logit link for the binomially distributed response or quasi-binomially distributed response, with treatment (i.e., control or exposure), species, and their interaction as fixed effects. We included body mass and length as additional fixed effects to account for potential differences in size between the control and exposure groups. The effect of treatment on survival probability was analysed in a generalized linear model with a logit link for (i) a binomially distributed response, and (ii) quasi-binomially distributed response:

(i) $$\mathrm{P}_i \sim Bin(1, p_i), logit(p_i) = \alpha + \beta_1 \mathrm{T}_i + \beta_2 \mathrm{S}_i + \beta_3 \mathrm{T}_i \times \mathrm{S}_i + \beta_4 \mathrm{M}_i + \beta_5 \mathrm{L}_i,$$

(ii) $$\mathrm{P}_i \sim Bin(1, p_i), logit(p_i) \sim N(\alpha + \beta_1 \mathrm{T}_i + \beta_2 \mathrm{S}_i + \beta_3 \mathrm{T}_i \times \mathrm{S}_i + \beta_4 \mathrm{M}_i + \beta_5 \mathrm{L}_i, \sigma^2),$$

where P_i corresponds to the binary survival (0: died, 1: survived) of the *i*th individual. α is the overall intercept, T_i the factor representing the treatment (i.e., control or exposure), S_i the species (i.e., starfish, serpent star, whelk, sea mouse, hermit crab, or flying crab), M_i the body mass, and L_i the body length of the *i*th individual and σ represents the dispersion parameter.

Model output of the generalized linear model with a binomially distributed response (i) is shown in **Table S3.6A** and in **Table S3.6B** for the generalized linear model with a quasi-binomially distributed response (ii). For the fixed effects, we only report the intercept, as well as the fixed effects that were kept constant at the overall mean during the multiple comparison procedure (i.e., body mass and length) in the first sub table. Species-specific comparisons of survival between the treatments were subsequently performed using a multiple comparison procedure. The fixed effects of main interest (i.e., treatment, species, and treatment × species) are given in the second sub table, as the output of the multiple comparison procedure. Significance codes: $p \leq 0.001$ ***, ≤ 0.01 **, ≤ 0.05 *, > 0.05 n.s.

Because the data were found to be underdispersed ($\varphi = 0.39$) for the binomial model, we switched to a generalized linear model with a quasi-binomially distributed response which accounts for observed dispersion. Underdispersion indicates lower variability in the data than expected, which can occur for instance when measurements are not fully independent (Kokonendji, 2014; Xekalaki, 2015). For survival experiments this could be due to, for example, neighbour or downstream effects when housing animals in the same water system. Accounting for dispersion did not cause overfitting, nor did it change the regression parameter estimates and thus the fitted values (**Table S3.6A**, **B**), but increased the sensitivity of the test.

Table S3.6A. Fixed effects and multiple comparisons explaining variation in animal survival, estimated in a generalized linear model with a binomial distribution. No significant effect of treatment was found. No mortality occurred in both treatment groups of starfish, whelk, and sea mouse.

Fixed effects						
	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	2.5310	1.5160	475	1.670	0.0950	n.s.
body mass	0.0987	0.0884	475	1.117	0.2640	n.s.
body length	−0.8756	0.6885	475	−1.272	0.2035	n.s.

Multiple comparisons							
Species	**Number of animals**		**Estimate**	**Standard error**	***z*-score**	***p*-value**	**Sig.**
	Control	**Exposure**					
Starfish	44	41	0.2562	3705.6730	0.000	1.000	n.s.
Serpent star	21	21	−0.2481	0.8390	−0.296	1.000	n.s.
Whelk	46	41	−0.0395	3605.9539	0.000	1.000	n.s.
Sea mouse	44	42	0.0486	3773.5626	0.000	1.000	n.s.
Hermit crab	43	43	−0.4444	0.8160	−0.545	0.995	n.s.
Flying crab	46	44	1.1987	0.5382	2.227	0.146	n.s.

Table S3.6B. Fixed effects and multiple comparisons explaining variation in animal survival, estimated in a generalized linear model with a quasi-binomial distribution. Only in flying crab a significant effect of treatment was found, where survival probability of animals in the exposure group was significantly higher than the control group. No mortality occurred in both treatment groups of starfish, whelk, and sea mouse.

Fixed effects						
	Estimate	**Standard error**	***df***	***t*-value**	***p*-value**	**Sig.**
intercept	2.5310	1.0314	475	2.454	0.0145	*
body mass	0.0987	0.0601	475	1.642	0.1013	n.s.
body length	−0.8756	0.4684	475	−1.869	0.0622	n.s.

Multiple comparisons							
Species	**Number of animals**		**Estimate**	**Standard error**	***z*-score**	***p*-value**	**Sig.**
	Control	**Exposure**					
Starfish	44	41	0.2562	2521.2162	0.0000	1.0000	n.s.
Serpent star	21	21	−0.2481	0.5708	−0.435	0.9986	n.s.
Whelk	46	41	−0.0395	2453.3706	0.0000	1.0000	n.s.
Sea mouse	44	42	0.0486	2567.4060	0.0000	1.0000	n.s.
Hermit crab	43	43	−0.4444	0.5552	−0.800	0.9633	n.s.
Flying crab	46	44	1.1987	0.3662	3.273	0.0064	**

To retrospectively test whether the sample size was sufficiently large to find an effect of certain size, we performed a power analysis with the `pwr.f2.test` function in the `pwr` package (Champely, 2020) with u = 13, v = 462, `sig.level` = 0.05 and we changed the values of argument "`f2`". In this function, "`u`" is the numerator degrees of freedom (i.e., the number of coefficients in the model minus the intercept), "`v`" the denominator degrees of freedom (i.e., n – u – 1), "`sig.level`" the significance level, and "`f2`" is the effect size measure. As suggested by Cohen (1988), an `f2` of 0.35, 0.15, and 0.02 represents a large, medium, and small effect size respectively. In case of the survival model, when `f2` was set to 0.05, 0.04, 0.03, and 0.02, we had a power of 91%, 82%, 67%, and 45% respectively. Hence with current sample size and a rather small effect size, we find high probability of rejecting the null hypothesis if a true effect is present (i.e., an alternative hypothesis is true).

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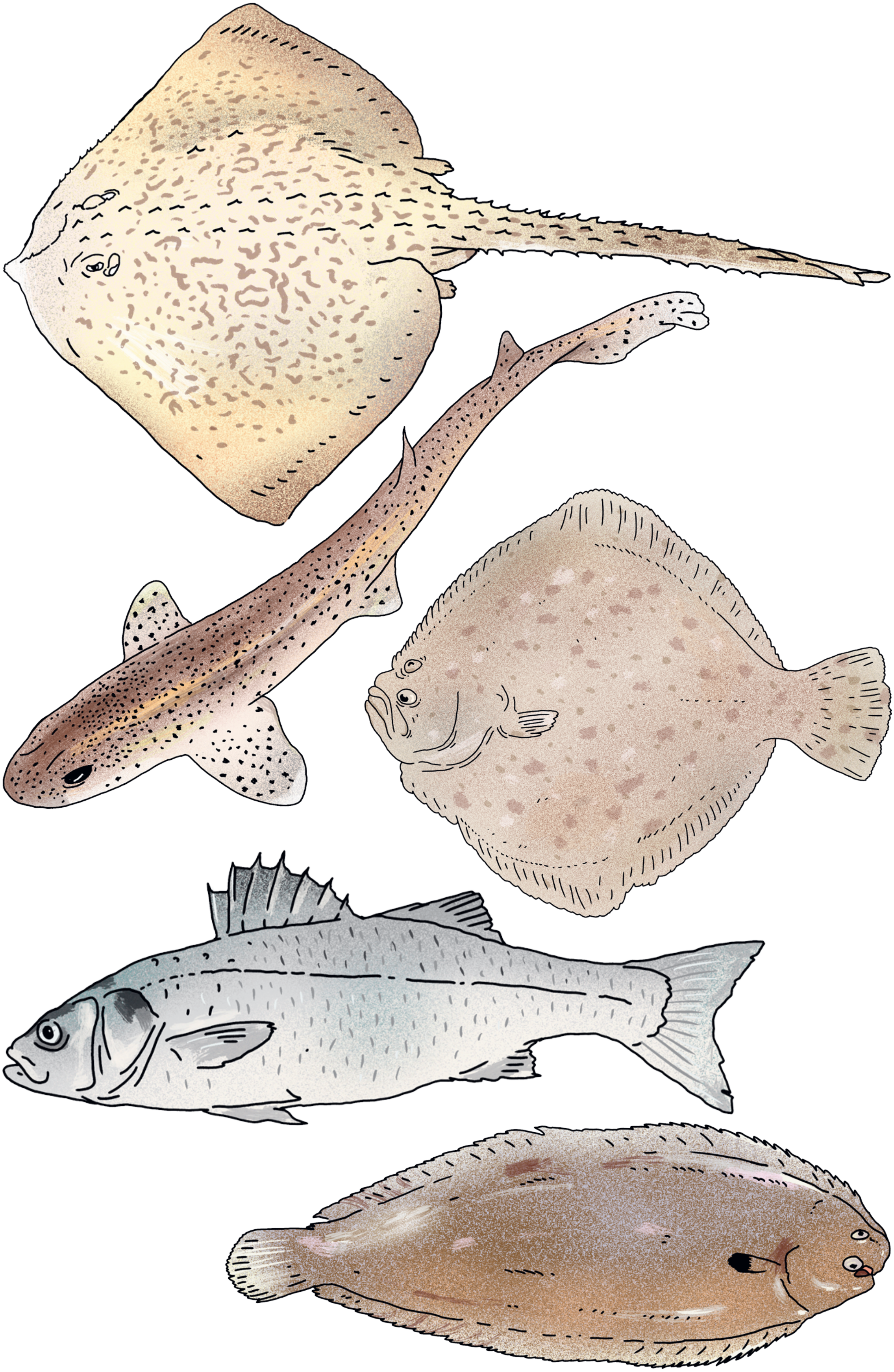

Chapter 4

Behavioural response thresholds of marine fish species for pulsed electric fields

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Abstract

Electrical pulse trawling is an alternative to conventional beam trawling for common sole (*Solea solea*), with substantially less discards, lower fuel consumption, and reduced impact on the benthic ecosystem. Pulsed electric fields are used to drive the fish from the seafloor and immobilise them in front of the nets. Concerns exist, however, that the electric fields may affect fishes outside the trawl track. Here, we address these concerns by measuring amplitude thresholds for behavioural responses and by comparing these response thresholds to simulated field strengths around fishing gear. Electroreceptive small-spotted catshark (*Scyliorhinus canicula*) and thornback ray (*Raja clavata*) as well as non-electroreceptive European seabass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*), and common sole were, one at the time, placed in a ⌀2.5 m circular tank with seven, individually controlled, evenly spaced electrode pairs, spanning the diameter of the tank. Behavioural responses were assessed from camera recordings for different pulse amplitudes and for different fish positions relative to the stimulating electrodes. Electrical stimulation consisted of a Pulsed Bipolar Current at 45 Hz and 0.3 ms pulse width, similar to those used in commercial gears. Computer simulation of the electric field, verified with *in situ* measurements, were used to determine the field strength at the location of the animal. Thresholds for different species varied between 6.0 and 9.8 V m^{-1}, with no significant difference between electroreceptive and non-electroreceptive species. The thresholds correspond to a distance of maximally 80 cm from the electrode arrays in simulated electric fields around commercial fishing gears. These findings suggest that electrical pulses as used in pulse trawling are unlikely to elicit a behavioural response outside the nets that surround the electrode arrays.

Keywords: bottom trawling; bycatch; electroreceptive fish; electrical pulse fishing; fish swimming; North Sea; staircase procedure; receiver-operating characteristic analysis

4.1 Introduction

Bottom trawling is widely used to capture demersal fish and invertebrates but comes with negative effects on the marine ecosystem and environment (de Groot, 1984; Jones, 1992; Bergman and van Santbrink, 2000; Paschen *et al.*, 2000; Kaiser *et al.*, 2006; Callaway *et al.*, 2007; Thurstan *et al.*, 2010; Poos *et al.*, 2013; Uhlmann *et al.*, 2014; Hiddink *et al.*, 2017; Amoroso *et al.*, 2018; McConnaughey *et al.*, 2020; Mazor *et al.*, 2021). Fishing gear innovation may help to limit negative environmental effects of bottom trawling (Brewer *et al.*, 2006; He, 2007; Suuronen and Sardà, 2007; Haasnoot *et al.*, 2016; ICES, 2020a; van Hoof *et al.*, 2020). For bottom trawls targeting common sole (*Solea solea*) in the North Sea, a promising modification is the replacement of tickler chains with electrode arrays (Soetaert *et al.*, 2015a). Whereas tickler chains use mechanical stimulation to chase the fish out of the sediment, pulse gears use pulsed electric fields to drive the fish from the seafloor and immobilise them in front of the nets (Soetaert *et al.*, 2015a, 2019). Fitting trawlers with electrode arrays can lessen the environmental impact by reducing fuel consumption (van Marlen *et al.*, 2014; Poos *et al.*, 2020), discard rates (van Marlen *et al.*, 2014), physical disturbance of the benthic ecosystem (Depestele *et al.*, 2016, 2019; Rijnsdorp *et al.*, 2020a, 2021a), and impact on benthic organisms (Soetaert *et al.*, 2015b, 2016a; Bergman and Meesters, 2020; Boute *et al.*, 2021). Furthermore, pulse trawling has increased selectivity (van Marlen *et al.*, 2014; Poos *et al.*, 2020), discard survival rates (van der Reijden *et al.*, 2017), and revenues (Batsleer *et al.*, 2016) compared to tickler-chain trawling. When catch volumes are limited by regulations, the lower ecological footprint as a result of reduced towing speed and increased catch efficiency is, arguably, a major advantage (Rijnsdorp *et al.*, 2020a).

Concerns exist, however, that the electric field extends well beyond the netting, potentially affecting fish outside the trawl track (Desender *et al.*, 2017; ICES, 2018; Quirijns *et al.*, 2018). Exposure to pulsed electric fields may cause different responses, depending on the field strength (e.g. Snyder, 2003; Soetaert *et al.*, 2015a, 2019). High field strengths may cause epileptic seizures and whole-body muscle contractions. Lower field strengths may induce involuntary muscle twitches and uncontrolled swimming. Moreover, if a fish would be able to sense low field strengths it could lead to complex changes in behaviour. Thresholds for the different types of responses are likely species-specific and may also vary with waveform parameters such as pulse duration and frequency (de Haan *et al.*, 2016; Desender *et al.*, 2016; Soetaert *et al.*, 2019). Pulse trawlers targeting common sole, however, use invariable electrical settings (ICES, 2020b), leaving electric field strength as the dominant variable.

Common sole – the target species for pulse trawlers – respond to high field strengths by curling their body upwards in a U-shape during which they are immobilised, without obvious damage (Soetaert *et al.*, 2015a, 2016b). Whole-body muscle contractions in non-target species, especially bilaterally-symmetrical round fish, may however lead to spinal fractures and internal haemorrhages, as studied in the laboratory (de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c, 2018) and under field conditions (van Marlen *et al.*, 2014; Soetaert *et al.*, 2016d). Whole-body muscle contractions occur at field strengths above 37 V m^{-1} in Atlantic cod (*Gadus morhua*) resulting in a 50% spinal probability in larger specimens at 80 V m^{-1} (95% CI: 60–110 V m^{-1}) (de Haan *et al.*, 2016). Such high field strengths occur only in close proximity of the stimulating electrodes (de Haan *et al.*, 2016; de Haan and Burggraaf, 2018), and are unlikely to affect fish beyond the boundaries of the nets which are at about 40–80 cm from the electrode arrays depending on the pulse gear type and number of arrays (above and sideways from the most lateral electrode arrays). Lower field strengths, however, may extend well beyond the nets and could potentially cause major behavioural changes. It is unknown, however, if fish can respond to low electric field strengths generated by pulse trawlers.

Especially elasmobranchs might sense the electric fields at a large distance from the source. Elasmobranchs have electroreceptors, the ampullae of Lorenzini, with extremely high sensitivity to electric fields (Dijkgraaf and Kalmijn, 1962, 1963; Murray, 1962; England and Robert, 2021). They use, a.o., electroreception to detect prey that is, for example, hidden in the sediment (Montgomery and Bodznick, 1999; Newton *et al.*, 2019). Desender *et al.* (2017) found that exposure of electroreceptive small-spotted catshark (*Scyliorhinus canicula*) to a high electric field strength did not result in impaired electroreception. De Haan *et al.*, (2009) reported variable responses of small-spotted catshark depending on distance to the electrodes, but no attempt was made to quantify this relationship, nor to quantify threshold strengths for minimal behavioural responses. In a freshwater electrofishing context, non-electroreceptive fish have been found to respond to electrical stimuli of low field strengths via a startle response or galvanotaxis (Taylor *et al.*, 1957; Snyder, 2003; Pottier *et al.*, 2020). Galvanotaxis is expected to be minimal in marine electrotrawling because bipolar pulses are used, the scale of operation is larger than in freshwater (Bary, 1956; Polet, 2010; Soetaert *et al.*, 2019), and – if it occurs – galvanotaxis is presumably outweighed by the gear towing speed of about 4–5 knots (2.1–2.6 m s^{-1}) (Poos *et al.*, 2020; Rijnsdorp *et al.*, 2020a). Startle responses or even attraction or repulsion could occur, however, if fish sense the electric field besides or above the electrode arrays. Here, we investigate behavioural response thresholds of marine fish to the pulsed electric field used by commercial pulse trawlers.

For the behavioural measurements, fish were placed in a large circular tank with seven, individually controlled, evenly spaced electrode pairs, spanning the diameter of the tank. Absence or presence of a behavioural response was assessed from camera recordings for different electrical stimulation amplitudes and for different positions of the fish relative to the stimulating electrodes. Any visible change in behaviour, e.g. changing speed or direction of swimming, during the stimulation period was scored as a response. To assess field strength thresholds, pulse amplitude and choice of stimulation electrodes was varied according to a staircase procedure (Treutwein, 1995; Leek, 2001). Computer simulation of the electric field, verified with measurements in the experimental setup, were subsequently used to determine the electric field strength at the animal's location.

We quantified response thresholds in two electroreceptive species, small-spotted catshark and thornback ray (*Raja clavata*) (Kalmijn, 1971), as well as three non-electroreceptive species, European seabass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*), and common sole. These species represent round fish and flatfish as well as bycatch and target species of the pulse-trawl fishery, inhabit areas trawled by pulse vessels (Heessen *et al.*, 2015), and *S. canicula*, *D. labrax* and *S. solea* have previously been studied in electrotrawling research, albeit with a focus on injuries (Soetaert *et al.*, 2016b, 2018; Desender *et al.*, 2017). Although many fish species with different body shapes and sizes may encounter pulse trawls, our aim was to quantify individual and species-specific behavioural thresholds and compare electroreceptive with non-electroreceptive species to make a first assessment of behavioural sensitivity differences. To translate the measured response thresholds to a behavioural 'safety zone' around commercial fishing gear, we compare threshold field strengths to simulation of the electric field around the fishing gear. This way, we can estimate the distance relative to commercial gears at which fish may be impacted by the electric fields.

4.2 Materials and methods

4.2.1 Experimental animals and housing facilities

Small-spotted catshark were caught using short (~20 min) fishing hauls with a beam trawl by the RV Belgica. Thornback ray were caught by commercial beam trawlers. They were collected on the North Sea by scientists of the Flanders Research Institute for Agriculture, Fisheries and Food (ILVO) and transported to the ILVO laboratory facilities in Ostend (Belgium). After at least five weeks, animals were transported to the Carus Aquatic Research Facility of Wageningen University in Wageningen (the Netherlands). Turbot were caught by commercial pulse trawlers on the North Sea and first housed at Wageningen Marine Research location Yerseke (the Netherlands) and after at least five weeks transported to Wageningen. European seabass were acquired from the aquacultured stock at Écloserie Marine de Gravelines Ichtus (Gravelines, France) and common sole were acquired from the aquacultured stock of Stichting Zeeschelp (Kamperland, the Netherlands).

All fish were housed in a climate-controlled room in tanks containing aerated, artificial seawater (demi water with Aquarium Systems Reef Crystals sea salt) in closed circulation systems, at a 12:12 light regime. Water quality parameters were monitored daily, including ammonium, nitrite, and nitrate (MQuant) and pH, temperature, salinity, and conductivity (multi-parameter portable meter, MultiLine Multi 3630 IDS). Water was partially changed when ammonium, nitrite, or nitrate levels exceeded 0.5, 0.15, or 100 mg L^{-1} respectively. Water temperature, salinity, conductivity, and pH were 15.6 ± 1.3 °C, 34.1 ± 1.6 ppt, 5.3 ± 0.2 S m^{-1}, and 7.6 ± 0.3 respectively (mean ± SD). Small-spotted catshark, thornback ray, common sole, and turbot were housed in tanks with sediment (0.5–1 mm grain size for the first three species and 4–8 mm grain size for the latter species) to allow for natural behaviour (Filer *et al.*, 2008; Gibson *et al.*, 2015; Greenway *et al.*, 2016). European seabass was housed in a cylindrical tank without sediment to prevent damage. Animals were acclimated to laboratory housing conditions at least one month prior to experimentation and were fed by hand, two-to-three times per week, also during the experimental period (**Supplementary material 4.1**).

Collection of fish and subsequent experimental procedures were approved by the Animal Welfare Body of Wageningen University, the Animal Ethics Committee of Wageningen University & Research, and the Dutch Central Authority for Scientific Procedures on Animals (application number AVD1040020184945; experiment number 2017.W-0080.001 and 2017.W-0080.002) as well as the ethical committee of ILVO (reference number EC 2018/322) and the Animal Welfare Service of the Flemish government (DWZ/WVdS/18/115/44). All experiments were done in the Netherlands.

4.2.2 Experimental setup

The experimental setup consisted of a circular tank, 2.5 m in diameter, filled with 25 cm artificial seawater, connected to an external filtration system (EHEIM type 2260 bucket filter). The bottom was covered with white ceramic tiles to enhance contrast between the animal and background. The tank was aerated between measurement sessions. Water quality parameters were monitored at the start and at the end of the experimental procedure and were found to remain stable and similar to the housing tanks. Ammonium, nitrite, and nitrate levels were 0.03 ± 0.1, 0.02 ± 0.02, and 13.8 ±

11.7 mg L^{-1} respectively. Water temperature, salinity, conductivity, and pH were 15.4 ± 1.3 °C, 34.6 ± 0.8 ppt, 5.3 ± 0.1 S m^{-1}, and 8.1 ± 0.1 respectively.

To record fish behaviour, we used a Basler ace acA2040-90um NIR camera (2048 × 2048 px; images binned 2 x 2 at 25 fps) with a Kowa C-mount LM8HC F1.4 8 mm lens and a ⌀55 mm UV filter (Hama), for protection against spray water and salt. The camera was placed centrally at 230 cm above the bottom of the tank. Four halogen floodlights lights (400 W each) at a height of ~2.5 m and at ~1.5 m from the side of the tank illuminated the experimental arena. To create a soft-box effect and minimise external disturbances, white cotton sheets were placed around and above the experimental setup.

Fourteen electrodes were evenly spaced around the circumference of the tank, at a distance of 115 cm from the centre. Each electrode consisted of a brass rod, 6 mm in diameter, insulated with 1 mm thick heat-shrink tubing apart from the 10 cm at the tip, placed in the middle of the water column. Electrical stimulation was generated between a single pair of opposite electrodes, depending on the location of the fish. Electric field strengths were varied by the choice of electrode pair and the amplitude of the generated pulses. The electrical stimulus was generated by a custom-made, computer-controlled pulse generator connected to a DC power supply (60 V, 5 A; TENMA 72-2940) and a 22000 µF capacitor (63 V; EPCOS B41560-A8229-M). Pulse timings and selection of the output electrodes were controlled by means of a National Instruments PCI-6221 data acquisition card (VHDCI front connection type), connected with a SHC68-68-EPM 2 m cable to a SCB-68A interface. Pulse amplitudes were determined by the voltage of the power supply, under computer control. Pulse generation and image acquisition were programmed in `Python` (Python Software Foundation, n.d.) in combination with `OpenCV`. For each stimulation, the program saved settings and timing of the stimulus as well as camera images at the start and end of the stimulus for precise measurements of the animals' location.

4.2.3 Electrical waveform properties and electric field in the experimental setup

The electrical stimulus consisted of a rectangular-shaped Pulsed Bipolar Current (Soetaert *et al.*, 2019) at a pulse frequency of 45 Hz and pulse width of 0.3 ms (2.7% duty cycle), similar to those used in pulse systems for targeting sole (e.g. Soetaert *et al.*, 2015b, 2015a, 2019; ICES, 2020b; pers. comm. Harmen Klein Woolthuis of HFK Engineering B.V.). We used a stimulus duration of 3 s to provide ample response time, which is about a factor of 2 longer than exposures to commercial fishing gears with 4 m long electrode arrays (Soetaert *et al.*, 2016c, 2019) towed at about 5 knots (van Marlen *et al.*, 2014; Depestele *et al.*, 2019; Poos *et al.*, 2020; Rijnsdorp *et al.*, 2020a).

To determine the local strength of the pulsed electric field in the experimental tank, we used the AC/DC package in COMSOL Multiphysics v5.4.0.246 (COMSOL Multiphysics®, n.d.) to numerically simulate the field for a single electrode pair (**Figure 4.1**). Water conductivity was set at 5 S m^{-1}, and steady-state field strengths were calculated for a 10 V pulse. Computer simulation was verified with measurements of local field strengths in the experimental setup (**Supplementary material 4.2**), and were used to determine the local electric field strengths at the start time of stimulation.

4.2.4 Experimental procedure

All experiments were performed in the same setup, for one individual at a time. A series of measurements consisted of 20–277 stimulations, lasting about 2–5 hours. Before each measurement series, electrodes were lightly sanded to remove any corrosion (Stewart, 1973). Animals were transferred from the housing tank to the experimental setup in a net, which was submerged in a water-filled transportation tub. The animals were acclimated to the experimental setup until displaying normal behaviour, with a minimum of 30 min.

To determine a threshold, we used a modified staircase procedure (Cornsweet, 1962; Treutwein, 1995; Leek, 2001). The procedure started with a 5 V stimulus on an electrode pair expected to give no response, based on pilot measurements for each species. After absence of a response pulse amplitude was increased by 5 V. When the fish showed a response but did not change position, we further increased the field strength until the fish moved to a new location, where we could start a new threshold assessment staircase. In case the maximum value of 60 V elicited no response, an electrode pair closer to the fish was chosen. In cases of doubt, e.g. for minimal changes of swimming speed of direction, we lowered stimulation strength to re-assess the threshold. The time between stimuli was at least 20 s in case no response was observed. After a response, we waited until the animal resumed normal behaviour, with a minimum of 30 s. Measurements stopped after at least ten response staircases were gathered or if prolonged swimming behaviour prevented reliable measurements. A measurement session was performed by one of two persons conducting the experiments. One person checked all responses from camera images afterwards. After measurements, animals resumed normal behaviours including feeding behaviour and egg deposition (small spotted catshark). Body length was either measured with a measurement board (Rabone Chesterman No 47R mounted on a measuring board) or from camera images (small-spotted catshark, thornback ray, and turbot). We measured standard length for European seabass, turbot, and common sole and total length for small-spotted catshark and thornback ray (**Supplementary material 4.3**).

The number of specimens available per species differed because of supply and housing-space limitations, and the use of some individuals in pilot measurements to design and optimise our experimental setup and measurement protocol. For the finalised protocol, we started measurements with seven small-spotted catshark, six thornback ray, nine European seabass, seven turbot, and seven common sole. Each specimen was used in only one measurement series conducted in a single day. For one small-spotted catshark and two turbot we aborted the measurement series due to prolonged high swimming activity making it too difficult to perform the measurements. These animals were excluded from subsequent analyses due to a lack of measurement points. Reliable threshold measurements were obtained for five up to nine specimens per species.

4.2.5 Linking fish location to electric field strength in the experimental setup

Photographs taken at the start of the stimulus were used to determine the location of the fish with respect to the active electrode pair in ImageJ (Schneider *et al.*, 2012). For the location of the fish, we used the point on the body closest to one of the active electrodes, corresponding to the highest field strength. Measured coordinates in pixels were transformed into actual world coordinates taking lens projection and refraction at the air-water interface into account. Hereto, a ruler spanning the diameter of the tank, was placed in the tank and digitised. For thornback ray, turbot, and common sole we used a depth of 1 cm from the bottom of the tank while for small-spotted catshark and European seabass we used a depth of 5 cm. The electrical field strength at the fish's location was determined based on numerical simulation of the electric field in the setup (**Figure 4.1**) at a resolution of 0.5 cm, scaled by the actual stimulus amplitude. Model simulation and linear scaling of field strengths with stimulation amplitude were verified by recordings of field strengths at different locations and for different stimulation amplitudes (**Supplementary material 4.2**).

4.2.6 Response-threshold estimation

Examples of the staircase sequence for an electroreceptive and non-electroreceptive species is shown in **Figure 4.2A**. Based on these raw data, we first estimated the probability of an individual to respond to an electrical stimulus by fitting the response (0 or 1) as a function of the electric field strength in a Bayesian Bernoulli generalized linear mixed model (i) (**Figure 4.2B**). The model estimates individual-specific intercepts and slopes on the electric field strength to quantify individual-specific response curves. Additional variables included stimulus number and observer identity as random

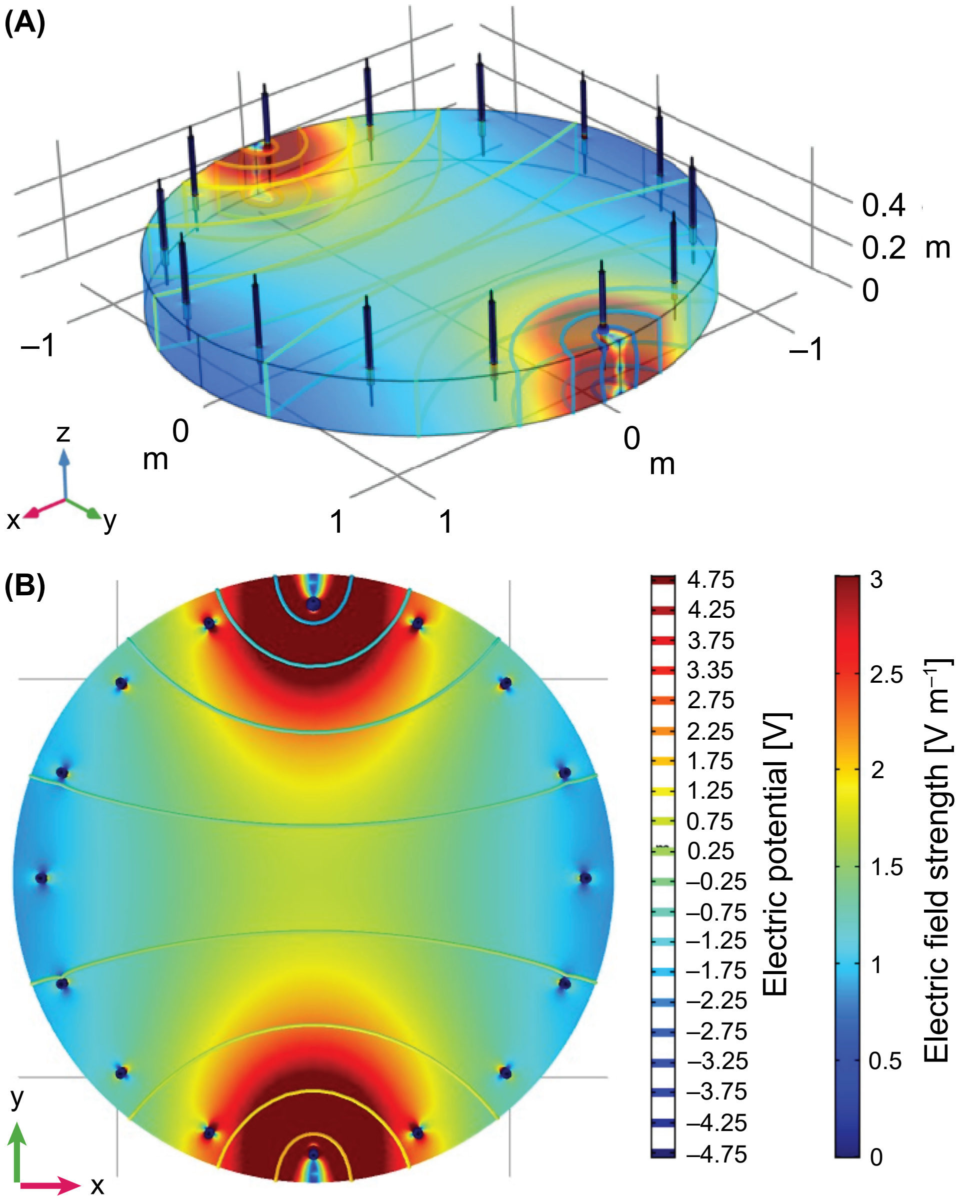

Figure 4.1. Schematic representation of the experimental setup in combination with the numerical simulation data of the electric field between one electrode pair showing the equipotential lines (–5 V to 5 V) and electric field strengths in V m^{-1}. All distances are shown in metres. **(A)** Three-dimensional view and **(B)** horizontal cross-section taken at 5 cm from the bottom (i.e. z = 0.05 m). Field strength values are clipped at 3 V m^{-1}.

intercepts to account for potential habituation to the stimulus, as well as between-observer variation, respectively:

(i) $$\mathrm{R}_{i,n} \sim Bin(1, p_{i,n}), logit(p_{i,n}) = \alpha_n + \alpha_o + \alpha_i + \beta_i \mathrm{E}_{i,n},$$

where $\mathrm{R}_{i,n}$ corresponds to the binary response (0: no response, 1: response) of the *i*th individual during the *n*th stimulus number, α_n to the stimulus-number-specific intercept, α_o to the observer-specific intercept, and α_i to the individual-specific intercept. $\mathrm{E}_{i,n}$ corresponds to the electric field strength experienced by the *i*th individual during the *n*th stimulus number. We used $N(0, 5^2)$ priors for the individual-specific intercepts and slopes on the electric field strength. For stimulus number and observer identity, we used a $N(0, \sigma^2)$ prior, each with the common standard deviation $\sigma \sim$ Student-$t_{(0, \infty)}(0, 10^2, 1)$. Three Markov Chain Monte Carlo (MCMC) simulation chains were run for 1,500,000 iterations, with a burn-in of 500,000 and a thinning of 1,000. Convergence was assessed by visual examination of the traces and by checking that $\hat{\mathrm{R}} < 1.01$.

Second, we performed a receiver-operating characteristic (ROC) analysis on the individual response curves to determine the optimal cut-off probability (optimal decision threshold) (Krzanowski and Hand, 2009). For this, we calculated the true positive rate (sensitivity), true negative rate (specificity), as well as the area under the ROC curve (AUC) for cut-off values ranging from 0 to 1 (**Figure 4.2C**, **D**). The AUC is a measure of the general model performance across all cut-off values (Krzanowski and Hand, 2009). The optimal cut-off response probability was then defined as the cut-off with the highest Youden Index, specifying the highest sum of sensitivity and specificity, as indicated by the arrows in **Figure 4.2D** and horizontal lines in **Figure 4.2B** (Krzanowski and Hand, 2009). For values above the optimal cut-off probability the model predicts a response, otherwise an absence of a response. The electric field strength threshold corresponds to the field strength at this optimal cut-off probability (dashed lines in **Figure 4.2B**). The above procedure was repeated for all MCMC samples of the estimated posterior distribution, to provide an individual-specific confidence interval of the cut-off probability, as well as of the corresponding field strength threshold. Response curves, ROC curves, and sensitivity and specificity as function of cut-off response probabilities for all specimens are listed per species in **Supplementary material 4.4**.

Finally, to compare response thresholds among species, individual-specific thresholds were fitted as a function of species and body length in a Bayesian Gaussian model (ii):

(ii) $$\mathrm{T}_i \sim N(\alpha + \beta_1 \mathrm{S}_i + \beta_2 \mathrm{L}_i, \sigma^2),$$

where T_i corresponds to the posterior mean response threshold of the *i*th individual, α to the overall intercept, S_i to the species, and L_i to the body length of the *i*th individual. σ is the residual standard deviation. The inclusion of body length enables us to estimate species-specific response thresholds that are independent of within-species variation in body length. We used $N(0, 5^2)$ priors for all fixed effects (i.e. species and body length) and the same MCMC specifications and convergence assessment as for model (i).

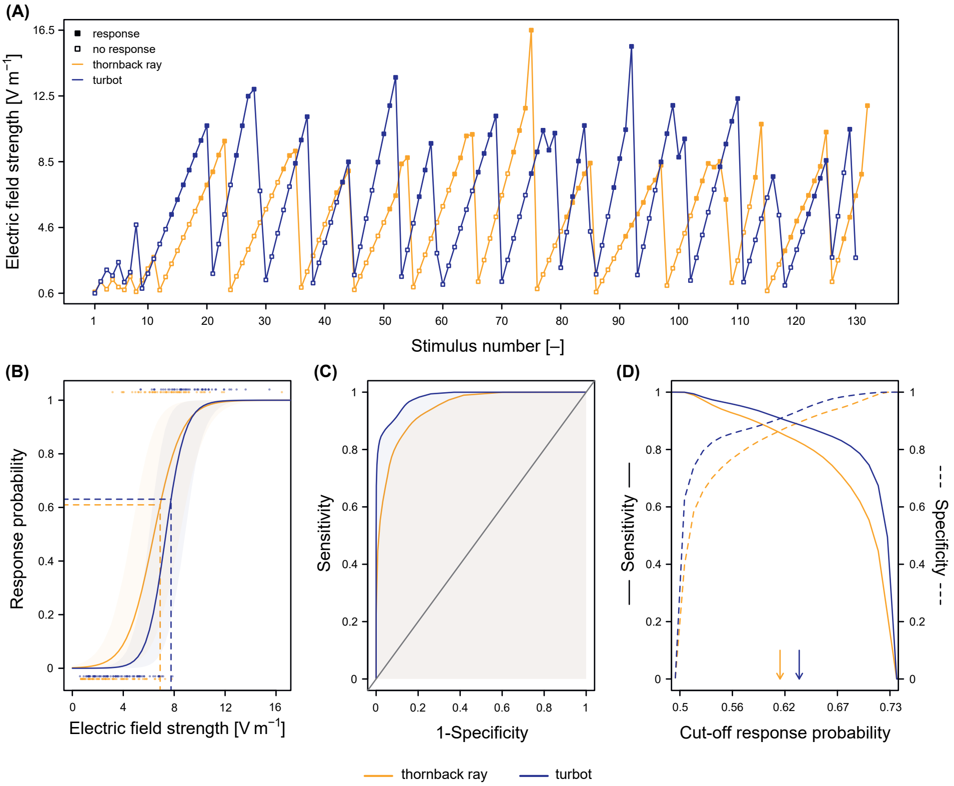

Figure 4.2. Individual responses to the electrical stimulus and corresponding receiver-operating characteristic (ROC) analysis for an example electroreceptive (thornback ray; orange) and non-electroreceptive (turbot; blue) species. **(A)** Staircase sequence of stimulus strengths with the corresponding responses (open and filled squares for absence or presence of a response respectively). **(B)** Response probability (± 95% CI) as a function of electric field strength (individual response curve). Dots at the bottom and top indicate the raw data for absence or presence of a response respectively as shown in **(A)**. Horizontal dashed lines indicate the optimal cut-off probability determined using the ROC-analysis shown in **(D)**. The vertical dashed lines indicate the corresponding mean electric field strength threshold. **(C)** ROC curve or true positive rate (sensitivity) as a function of false negative rate (1−specificity). General model performance was calculated as the area under the ROC curve (AUC; shaded area). **(D)** True positive rate (sensitivity) and true negative rate (specificity) as function of cut-off response probability. The optimal cut-off response probability was defined as the cut-off with the highest Youden Index, specifying the highest sum of sensitivity and specificity (arrows).

The difference in response thresholds between electroreceptive and non-electroreceptive species was quantified by pooling the posterior estimates of the species-specific response thresholds for electroreceptive and non-electroreceptive species together. We subsequently calculated the difference between a random posterior sample ($n = 1{,}000$) of each category.

All response-threshold analyses were carried out in a Bayesian framework using the `JAGS` package (Plummer, 2003) in `R` v4.1.2 (R Core Team, 2021). The models were fitted using the `rjags` and `R2jags` packages (Plummer, 2019; Su and Masanao, 2020).

4.2.7 Linking response thresholds to commercial fishing gear

To compare measured thresholds to the electric field strength around a pulse trawl gear and determine a maximum distance from electrode arrays at which fish species may be affected, we simulated the electric field around a pair of electrode arrays as used in pulse trawling for common sole (**Figure 4.3**) (COMSOL Multiphysics®, n.d.). Pulse gear electrode arrays run in parallel from the pulse modules attached to the beam or PulseWing (Delmeco Group B.V. or HFK Engineering B.V. pulse gear manufacturer respectively) to the ground rope of the net (Soetaert *et al.*, 2019; Rijnsdorp *et al.*, 2021a). Electrode arrays consist of connected conductive parts of 12.5 cm long and ⌀3 cm, separated by 22 cm long, insulated parts (Soetaert *et al.*, 2019). Large trawlers have 24–28 electrode arrays over a beam width of 12 m, small trawlers typically have 10 electrode arrays over a beam width of 4.5 m (Depestele *et al.*, 2016; Rijnsdorp *et al.*, 2020b, 2021a). Neighbouring electrode arrays act as an anode-cathode pair. All pairs are activated at the same frequency, but pulses are alternated in time and, therefore, different pairs do not interact. To describe the electric fields around a pulse gear, simulating one electrode array pair suffices. We simulated a pair of electrode arrays 41.5 cm apart, similar to the distance in commercial gears (Soetaert *et al.*, 2019). The electrode arrays were placed at the interface between water ($5\ \mathrm{S\ m^{-1}}$) and sediment ($0.5\ \mathrm{S\ m^{-1}}$). Steady-state electric field strengths were determined for a stimulation peak of 60 V, similar to the maximum voltage setting for commercial gears.

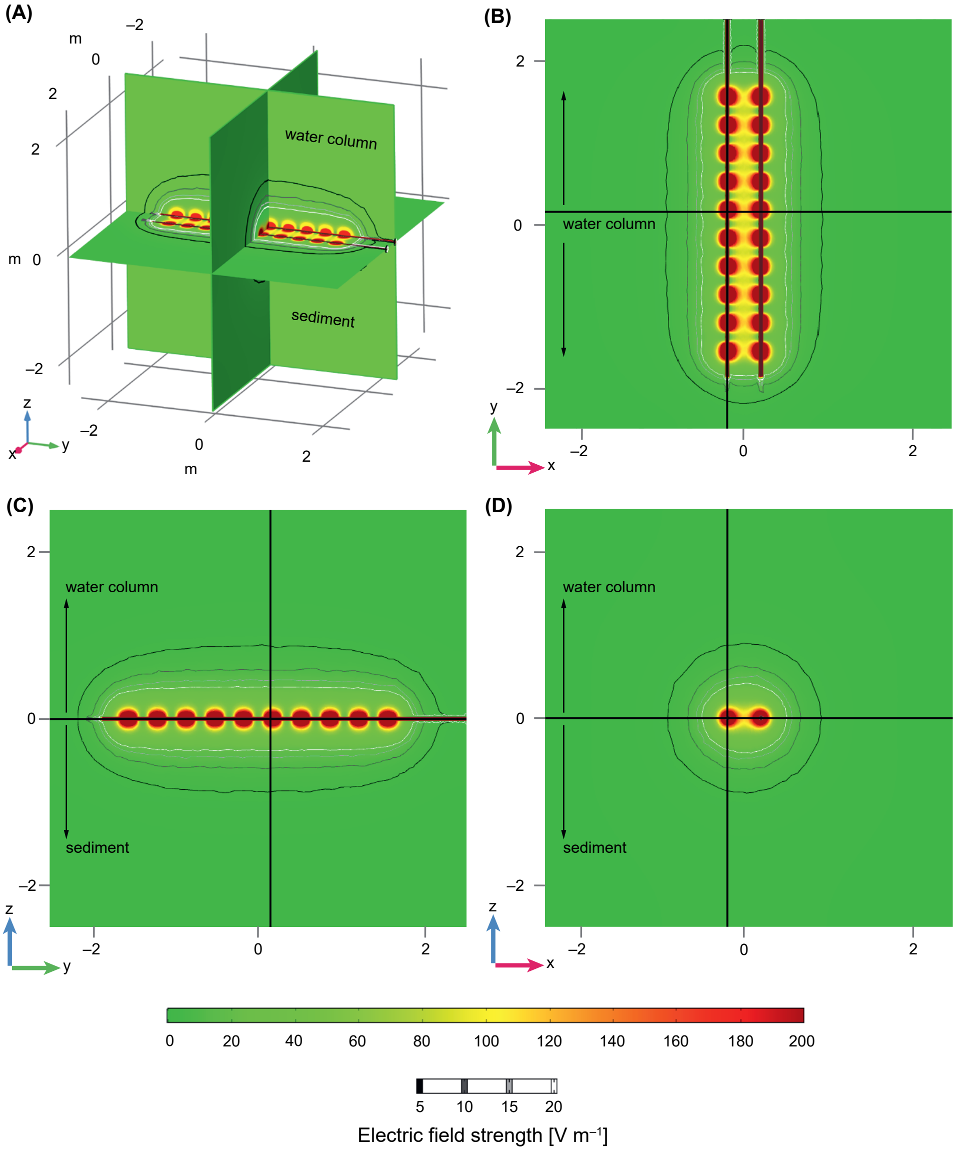

Figure 4.3. Contour plots with the numerical simulation data of the electric field strengths around a pair of commercial electrode arrays separated at 41.5 cm and computed at a steady-state of 60 V. All distances are shown in metres. **(A)** Three orthogonal planes through the electrode array pair. These views are shown in panels **(B)**, **(C)**, and **(D)** where the thick black lines indicate the locations of the planes shown in the other panels. **(B)** Horizontal cross-section at z = 0. **(C)** Vertical cross-section through one of the electrode arrays. **(D)** Vertical cross-section orthogonal to the electrode arrays. Field strength values close to the electrodes are clipped at 200 V m^{-1}. Contour lines indicate equal field strengths at 5, 10, 15, and 20 V m^{-1} (thin lines from black to white). Positive and negative z-values are the water column and sediment respectively.

4.3 Results

Responses varied from a full escape by a swimming bout to minimal movements of body parts that were closest to the electrode. Flatfish seemed more likely to remain stationary after responding to the electric field in contrast to small-spotted catshark and European seabass, which had the tendency to start swimming when responding. In general, however, animals showed similar behavioural patterns throughout a recording session.

4.3.1 Behavioural response thresholds

General model performance across all cut-off values estimated as the area under ROC-curve (AUC) was high for all individuals at 0.95 ± 0.03 (mean ± SD; **Supplementary material 4.5**). The effect of habituation on the response of an individual to the electrical stimulus was negligible ($\alpha_{\text{post.mean}} = 0.000$; 95% CI: −0.204–0.243). Likewise, there was no significant difference in the estimated response probability between the observers ($\alpha_{\text{post.mean}} = 1.931$, $P_{\text{MCMC}} = 0.336$). The estimated individual-specific electric field strength thresholds varied between 3.4–14.9 V m^{-1} with a mean of 7.3 V m^{-1} (95% CI: 4.1–12.4) (**Figure 4.4**). The mean within-individual variation of the estimated field strength thresholds was relatively small (SD of 0.13 V m^{-1}) compared to the mean between-individual variation (SD of 2.36 V m^{-1}). Body length did not significantly influence the individual-specific field strength thresholds ($\beta_{\text{post.mean}} = 0.067$, $P_{\text{MCMC}} = 0.285$).

Thresholds (mean; 95% CI) were lowest for the non-electroreceptive European seabass (5.9 V m^{-1}; 4.7–7.1) and common sole (6.0 V m^{-1}; 4.5–7.4), and highest for the electroreceptive small-spotted catshark (9.8 V m^{-1}; 8.2–11.4). The electroreceptive thornback ray (7.6 V m^{-1}; 6.0–9.1) and non-electroreceptive turbot (8.4 V m^{-1}; 6.6–10.1) showed intermediate sensitivities (**Figure 4.4**). Although thresholds were in the same range, pair-wise comparisons of the field strength thresholds between species showed some significant results. Notably, small-spotted catshark was significantly less sensitive than European seabass ($\beta_{\text{post.mean}} = -3.929$, $P_{\text{MCMC}} = 0.001$) and common sole ($\beta_{\text{post.mean}} = -3.867$, $P_{\text{MCMC}} = 0.001$). Furthermore, turbot was significantly less sensitive than European seabass ($\beta_{\text{post.mean}} = -2.458$, $P_{\text{MCMC}} = 0.027$) and common sole ($\beta_{\text{post.mean}} = -2.397$, $P_{\text{MCMC}} = 0.037$) (**Figure 4.4**). However, we found no significant difference in sensitivity between electroreceptive and non-electroreceptive species ($\beta_{\text{post.mean}} = 1.921$, $P_{\text{MCMC}} = 0.332$).

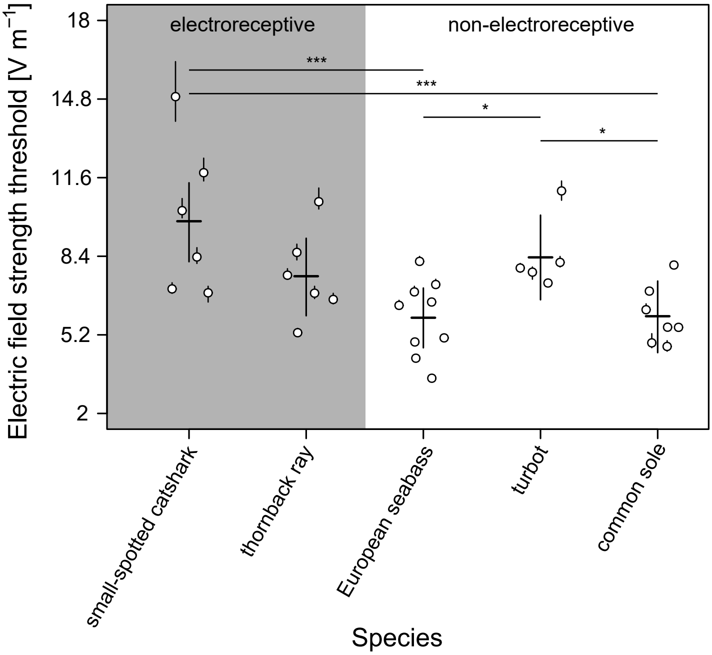

Figure 4.4. Mean individual-specific (open circles) and species-specific (horizontal line segments) electric field strength thresholds (plus 95% CI) for a behavioural response. Species-specific thresholds were predicted for the species-specific mean body length (small-spotted catshark = 58 cm; thornback ray = 51 cm; European seabass = 30 cm; turbot = 27 cm; common sole = 30 cm), and thus, accounts for within-species variation in body length. Small-spotted catshark had a significantly higher electric field strength threshold compared to European seabass and common sole. Turbot had a significantly higher response threshold compared to European seabass and common sole. We found no significant difference in response threshold between electroreceptive and non-electroreceptive fish. Significance codes: $P_{MCMC} \leq 0.001$ ***; $0.01 < P_{MCMC} \leq 0.05$ *.

4.3.2 Fish response thresholds related to the pulse-trawl gear

The electric field around a commercial electrode array pair of a pulse trawl gear is heterogeneous, with highest field strengths close to the electrodes (**Figure 4.3** and **4.5**). Electric field strength quantifies the gradient in voltage (V m^{-1}) and determines the current for a specified conductivity of the medium. The electric field strength, as created by the source voltage, is proportional to the voltage and is inversely related to the square of the distance from the source. The electric field shape created by an electrode array pair is a complex function of the size and shape of the electrodes, conductivity of the medium, and the spatial layout of the electrodes (**Figure 4.3** and **4.5**). Electric field strengths are similar in the water column and in the sediment and are largely independent of the conductivity of the sediment (**Figure 4.3** and **4.5**). Close to the electrode, electric field strengths reach values of 200 V m^{-1} and show a strong modulation along the length of the array, with high values close to the electrodes and lower values near the insulators. At larger distances, modulations in the longitudinal directions disappear. Field strengths drop below a value of 10 V m^{-1} at a distance of about 30 cm from the electrode and below 5 V m^{-1} at about 50 cm (**Figure 4.5**). This decline is slightly steeper in the lateral direction than in the vertical direction.

To estimate the threshold distance of the fish relative to the electrode pair, we quantified the electric field strengths along the horizontal and vertical axis and compared these with our behavioural thresholds. Based on the mean behavioural response thresholds of about 6–10 V m^{-1}, the maximum distance at which studied species are expected to show a response is about 60 cm to an electrode in the lateral direction and 80 cm in the vertical direction, with little interspecies variation.

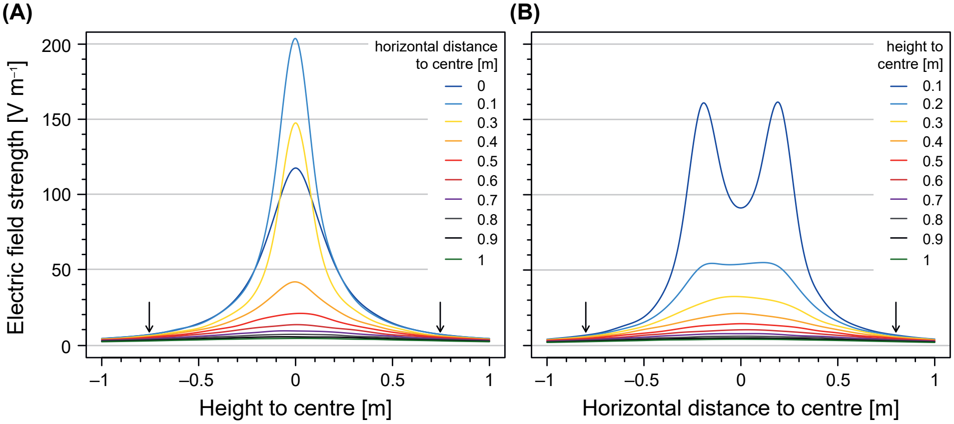

Figure 4.5. Electric field strengths as a function of **(A)** height relative to the seabed (z-axis in **Figure 4.3**) for different horizontal positions relative to the centre of an electrode pair (along the x-axis in **Figure 4.3**). Electric field strengths as a function of **(B)** horizontal distance to the centre of an electrode pair (x-axis in **Figure 4.3**) for different heights above the electrodes in the water column (z-axis in **Figure 4.3**). The electrode pair is situated at the interface between water column and sediment (i.e. height of 0 m) and at 41.5 cm apart (i.e. the electrode cores at –19.25 and 19.25 cm on the horizontal distance to the centre). Sections through the electrodes were left out because zero field strength values in the electrodes and high values around the electrode distorted the view on low values in which we are interested. The horizontal-distance-to-centre value 0.2 m and height value 0 m are therefore not shown in panel **(A)** and **(B)** respectively. The arrows indicate the mean fish response threshold of 8 V m^{-1}.

4.4 Discussion

Understanding fish behaviour in response to fishing gear deployment can provide valuable insights into e.g. capture mechanisms and selectivity (Wardle, 1986; Fernö, 1993; Winger *et al.*, 2010; Gibson *et al.*, 2015; Robert *et al.*, 2020). Pulse trawlers use electric fields as main stimulus, which extend beyond the mechanical disturbance and physical boundaries of the gear. Here, we addressed concerns regarding the potential effect of the pulsed electric field around the fishing gear on the behaviour of marine fishes. We quantified response thresholds of five marine fish species, two electroreceptive and three non-electroreceptive, for electric field strengths under controlled laboratory conditions. These thresholds were subsequently compared to the numerically-simulated electric field around a commercial electrode array pair to determine a safety zone where fish are expected not to respond to the field.

4.4.1 Behavioural responses

We observed various responses during electrical stimulation, ranging from small fin movements to swimming bouts and whole-body muscle contractions. Our findings show that fish can respond to the electric field before encountering high, immobilising field strengths. Observed behavioural responses corroborate previous results for small-spotted catshark by de Haan *et al.* (2009), who reported shortly closing an eye and contraction of body parts closest to the active electrode. In a few instances, we observed whole-body muscle contractions similar to those reported in small-spotted catshark, European seabass, and common sole exposed to high electric field strengths (Stewart, 1977; de Haan *et al.*, 2009; Soetaert *et al.*, 2016b, 2018; Desender *et al.*, 2017). For common sole, the observed bending of the body during exposure to higher field strengths resembles the Omega jump, described by (Kruuk, 1963) as an escape from the sediment. Body bending during high-frequency, high-field-strength electrical stimulation was also observed in dab (*Limanda limanda*) (de Haan *et al.*, 2015), European plaice (*Pleuronectes platessa*), European flounder (*Platichthys flesus*), brill (*Scophthalmus rhombus*), and lemon sole (*Microstomus kitt*) (Stewart, 1977) and is presumably the consequence of asymmetrical muscle distribution in flatfish between the eyed and blind side. In the few instances of relatively high field-strength exposure during our measurements, turbot would bend only slightly and thornback ray would fold/curl its wings. In European seabass and turbot, we also observed distended opercula and protrusion of the mouth indicating that the axial swimming muscles, which are used by fish for suction feeding (Camp *et al.*, 2015; Jimenez and Brainerd, 2020), contract during electrical pulsing.

Besides our observations during the threshold measurements, we noticed various other phenomena. Small-spotted catsharks deposited (fertilised) eggs before and after the experimental procedure, as described by de Haan *et al.* (2009) during the post-exposure period, but not by Desender *et al.* (2017). Our experimental animals resumed normal feeding after exposure and showed no mortality. Similar findings, with a few exceptions, have been reported for small-spotted catshark, European seabass, common sole, and

Atlantic cod exposed to high electric field strengths (de Haan *et al.*, 2009, 2016; Soetaert *et al.*, 2016c, 2016b, 2018; Desender *et al.*, 2017).

4.4.2 Behavioural response thresholds

Behavioural response thresholds for the studied species varied between 6.0 and 9.8 V m^{-1}. To our knowledge, these are the first quantitative behavioural threshold values reported in the context of marine electrotrawling for common sole. Although de Haan *et al.* (2016) reported that Atlantic cod did not respond when exposed to a field strength of 4 V m^{-1} but showed muscle contractions at values of ≥37 V m^{-1}, step sizes were not sufficient to quantify a behavioural response threshold. Few studies have examined the behavioural response of marine fishes to low electric field strengths as the focus has generally been on galvanotaxis/electrotaxis (Bary, 1956; Diner and Le Men, 1971, 1974; Klima, 1972; Polet, 2010; D'Agaro, 2011), immobilising whole-body muscle contractions referred to as electronarcosis, tetanus, or cramp (Bary, 1956; Diner and Le Men, 1971, 1974; Stewart, 1977; Polet, 2010; de Haan *et al.*, 2016), and (internal) injuries (de Haan *et al.*, 2016; Desender *et al.*, 2016; Soetaert *et al.*, 2016c, 2016b). Bary (1956) studied 'minimum response values' based on body jerks during stimulation in golden grey mullet (*Chelon auratus*), European flounder, and European seabass of different body lengths, but in a homogeneous electric field with waveforms different from those used in pulse trawling for common sole. Stewart (1975), however, recognised the relevance of these thresholds and evaluated the selectivity of an electrode array system that is towed perpendicularly with respect to the seafloor. Hereto, he converted the golden grey mullet data, expressed in voltage gradient along the fish body, from Bary (1956) to electric field strengths. For a 'minimal response threshold', he reported values of about 5 V m^{-1} with a limited fish-length effect. He subsequently related this threshold to the heterogeneous electric field and reported high reaction probabilities for fish close to the arrays. Although, no response distances with respect to the gear are presented, Stewart (1975) demonstrates the use of response thresholds in gear-selectivity assessment.

We quantified behavioural response thresholds for five marine fish species and found some significant interspecies differences that could not be explained by body length. It should be noted though that intraspecies length variation was limited, and thus, we have to be cautious when concluding that body length does not affect behavioural response thresholds. Furthermore, these species do not only differ in size, but also in a multitude of other anatomical and morphological properties such as body shape, which may explain some of the interspecies differences in behavioural response thresholds (Emery, 1984). Our main objective was to estimate threshold sensitivities of different species, flatfish and round fish, target and non-target, electroreceptive and non-electroreceptive, of representative sizes. These measured sensitivities provide a first estimate of the distance over which similar fish could be affected around a pulse gear. Future studies should try to increase sample sizes with larger intraspecies and interspecies variation in body length, to identify the sources of the observed interspecies differences in behavioural response thresholds.

4.4.3 Elasmobranch frequency-response characteristics for electroreception

We found no sensitivity difference between the electroreceptive (small-spotted shark and thornback ray) and non-electroreceptive species (European seabass, turbot, and common sole). Electroreceptive fish with ampullary receptors are highly sensitive for electric-field-strength amplitudes as low as 1 x 10^{-7} V m^{-1} (Dijkgraaf, 1963; Kalmijn, 1966, 1982; Tricas and New, 1998; Peters *et al.*, 2007) but only for a specific frequency range of about 0.1–25 Hz with a maximum sensitivity at around 1–8 Hz (Kalmijn, 1974; Montgomery, 1984; Peters and Evers, 1985; Collin, 2010; Crampton, 2019). Optimal frequency sensitivity of thornback ray ampullae of Lorenzini neurons is at 4 Hz with a relative steep drop in gain at higher frequencies and a gradual fall at lower frequencies to 0.05 Hz (Montgomery, 1984). In small-spotted catshark, Peters and Evers (1985) quantified the frequency sensitivity of the primary afferent neurons (i.e. before the stimuli are processed by the brain) and the respiratory reflex (i.e. after brain processing). They find a higher frequency-sensitivity optimum for the neurons (5–8 Hz with a fall-off of 2.3 and 3.4 dB $octave^{-1}$ at the low and high frequency side respectively) than for the respiratory reflex (0.1–1 Hz with a fall-off of 2.8 and 11.4 dB $octave^{-1}$ at the low and high frequency side respectively).

To understand why electroreceptive fishes are apparently insensitive for the pulsed electric fields in our study, one needs to take the frequency content of the stimulus into account. For pulses generated at 45 Hz, with a width of only 0.3 ms, most of the energy is in the high-frequency range. To analyse the mismatch in frequency content of the stimulus and the response characteristics of ampullae, we computed the frequency content of the electrical stimulus using the Fast Fourier transform function in `MATLAB` v9.5.0.944444 (MATLAB, 2018) (**Figure 4.6**). The pulse train stimulus in the time domain (**Figure 4.6A**, **B**) contains the fundamental frequency (45 Hz) and its odd integer harmonics series (**Figure 4.6C**). The amplitude spectrum shows that the frequency content largely mismatches the frequency sensitivity of the fish, with the larger part of the spectrum in the higher frequency range (>100 Hz), where electroreceptors have poor sensitivity. Although common-sole-targeting pulse trawlers may use lower frequencies down to 30 Hz (ICES, 2020b), the frequency content would still be well above the sensitivity range of the ampullae. In addition, the energy content is shifted to even higher frequencies, as each electrode array may participate in two pairs (except for the two most outer arrays). In this case, the effective frequency is doubled close to the electrodes, where electric field strengths are larger. When operating multiple electrode arrays using pulsed waveforms in close proximity, pulses may have different phases – depending on whether there is a centralised control – and thus create more complex waveforms with high frequencies in the area where the electric fields overlap (Beaumont, 2017; Soetaert *et al.*, 2019).

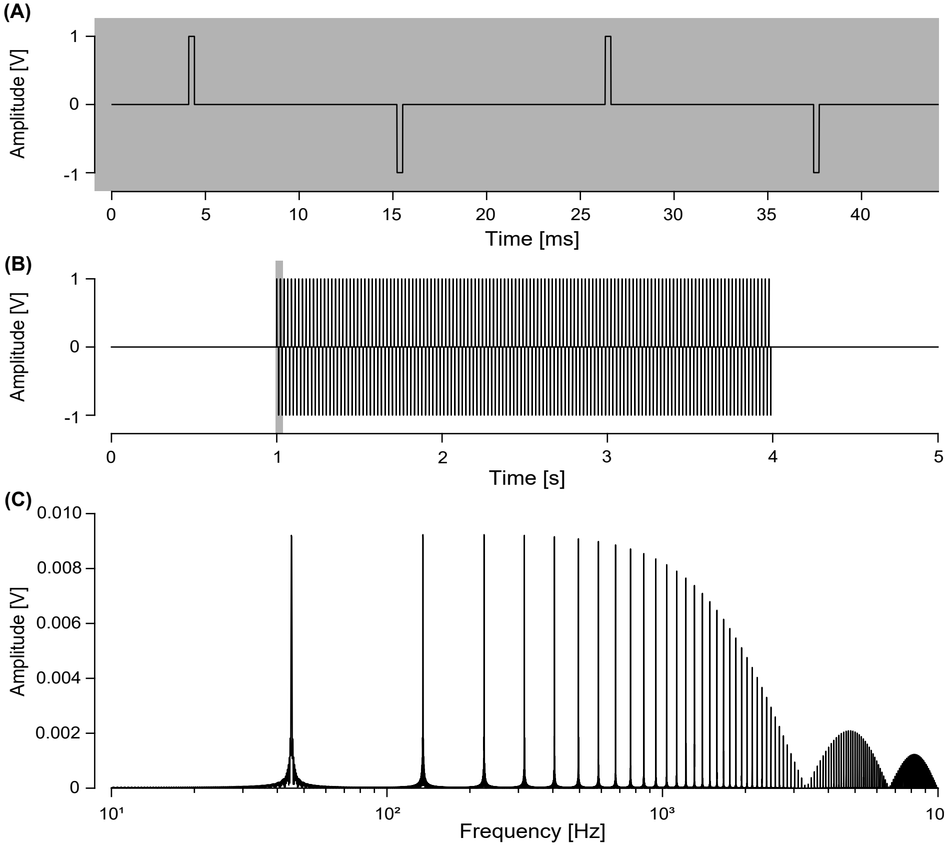

Figure 4.6. Characteristics of the square-shaped Pulsed Bipolar Current waveform offered at a pulse duration of 3 s. **(A)** Two cycles at 45 Hz, a pulse width of 0.3 ms, and an arbitrary amplitude of 1 V as well as **(B)** the full 135 cycles with two cycles shaded in grey. Pulse waveform is 45 Hz PBC (*PW* = 0.3 ms, *PB* = 10.81 ms) *sensu* Soetaert *et al.* (2019). **(C)** Fast Fourier transform spectrum of the waveform in **(B)** shown until 10,000 Hz.

The high-frequency limits for receptor and behavioural responses may thus explain the low sensitivity of the electroreceptive species to the pulsed electric fields. Moreover, the discrepancy between receptor and behavioural responses illustrates that a receptor response at high frequencies does not necessarily evoke a behavioural response. Even if the ampullae of Lorenzini would respond to the lower frequencies, the evoked neural response pattern may not match any pattern of interest to higher processing stages. The shape and size of the electric field could also affect the behavioural response as the ampullae of Lorenzini are tuned to detect bioelectric fields of e.g. small (buried) prey (Bedore and Kajiura, 2013), which have specific size characteristics (Kalmijn, 1982). Both the electric field in our experimental setup and the electric field around the pulse-trawl-gear electrode arrays are larger than that of a potential prey, thus likely unable to elicit a behavioural response of elasmobranchs. Based on the electrical waveform properties of the stimulus used by pulse trawls to catch common sole, in combination with the similarity of measured threshold sensitivities, it seems unlikely that electroreceptive species are more sensitive to the pulsed electric field than non-electroreceptive species.

4.4.4 Fish response thresholds related to the pulse-trawl gear

Our numerical simulation show that the electric field around commercial electrode arrays is heterogeneous, decreasing with increasing distance following the inverse-square law, which corroborates *in situ* measurements around various marine electro-array designs (Stewart, 1975, 1977; Polet *et al.*, 2005; Murray *et al.*, 2016) including those used to target common sole (de Haan *et al.*, 2016; de Haan and Burggraaf, 2018). The field shape in the vertical cross-section orthogonal to the electrode arrays (**Figure 4.3D**) is highly similar to an electrode-pair simulation of a brown-shrimp (*Crangon crangon*) electrotrawl (Verschueren and Polet, 2009). In addition, similar field strengths occur in the water column and sediment and are largely independent of the conductivity of the sediment, which corroborates measurements at various field locations (de Haan and Burggraaf, 2018) and in the laboratory (Murray *et al.*, 2016). Positioning of the arrays in the sediment was based on Depestele *et al.* (2019), who modelled penetration depth of an *in situ* electrode array to be between 1–1.5 cm. In our simulation, we computed the steady-state electric field at a peak amplitude of 60 V which is used as maximum setting for commercial gears. In field conditions, however, this value drops to 52–58 V at the seafloor, depending on the season (Rijnsdorp *et al.*, 2020b). Hence, we may overestimate the electric field strengths of our commercial gear simulation by 3–13% with respect to the in situ values at the seabed. Finally, different commercial electrode array design variations are expected to minimally affect electric field strength distributions around an electrode pair (see Soetaert *et al.* (2019) for an example of two electrode array designs).

However, as electrodes may be grouped or evenly spaced on the array, they will render a different pattern in time during towing such as a gated-burst-like exposure (de Haan *et al.*, 2011; Soetaert *et al.*, 2019). Future research should focus on the dynamic effect of towing and pulsing to study the generated waveforms patterns at different positions with respect to the electrode arrays.

Behavioural responses of studied species will be limited to 80 cm from the electrode arrays in a vertical direction and 60 cm in a horizontal direction for the most lateral electrode arrays, with respect to the centre of an electrode pair. The electrode arrays are surrounded by nets on the side and top starting at the beam or PulseWing (i.e. trawl opening) to prevent fish from escaping. The electrodes that generate the electric fields start at about 4 m after the trawl opening (Soetaert *et al.*, 2019). Consequently, a fish is trapped when entering the trawl opening: the only way to escape is through the meshes of the netting material or to outswim the trawl. If the fish fits through the meshes, both options are possible should the animal be able to detect the electric field before immobilisation. Fishes could outswim the trawl for a short period depending on the species, size, and water temperature (He, 1993; Videler and He, 2010), but the towing speed is generally too fast to outswim for a sustained period. In this case, behavioural response thresholds are only relevant if the fish could detect the electric field ahead of the electrodes in the trawl opening. Based on our findings, fishes are unlikely to respond to the electric field behaviourally when entering the trawl opening. We expect that, due to the towing speed, most, if not all, fishes will flow past the electrode arrays. Fish swimming in the vicinity of the gear could potentially be affected by the electric field extending beyond the nets around the electrode arrays. These nets, however, are placed at about 50–90 cm above the arrays and at about 40–80 cm laterally from the most outer arrays depending on the pulse-gear type and rigging (Rijnsdorp *et al.*, 2021a). Based on our data, fishes may respond behaviourally to the electric field at a maximum distance of 20–30 cm from the nets around the arrays. Finally, fishes may be buried in the sediment when the trawl passes, which is common for the target species of North Sea electrical pulse trawling, the common sole. This species is nocturnal and may be buried in the sediment during the day (Kruuk, 1963; de Groot, 1971). Although the lower sediment conductivity results in relatively lower internal field strengths compared to fish at a same distance in the water column because the conductivity of the sediment and fish will be more similar (ICES, 2020b), pulse trawl footrope selection showed a reduced diurnal effect compared to tickler-chain fishing (Rijnsdorp *et al.*, 2021b) indicating that the electrical stimulus is effective in removing fish from their buried position. Based on our findings, the effect of the electric field on fish behaviour – including buried common sole – is expected to be largely limited to the trawl gear width. This, however, does not exclude a response to an oncoming trawl via e.g. visual cues or vibrations from the gear and vessel engine (Arimoto *et al.*, 2010; Yan *et al.*, 2010; Jordan *et al.*, 2013).

In conclusion, our results indicate that marine fish show relatively high thresholds for a behavioural response to pulsed electric fields. Mean behavioural response thresholds across species varied between 6 and 10 V m^{-1}. Electroreceptive species were not more sensitive, which is presumably due to a mismatch in frequency content of stimulation and receptor sensitivity to low frequencies. These thresholds correspond to a distance of about 60–80 cm from electrodes in commercial pulse-trawl gears. Our findings indicate that behavioural responses of fishes to electrical pulses are largely restricted to the trawl path and hardly extent beyond the netting of pulse gear, with some variation per pulse-gear type and rigging.

4.5 Statements

4.5.1 Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

4.5.2 Author contributions

PGB, RPMP, and MJL contributed to the conception and design of the study; MJL programmed the software to run the pulse stimulator equipment with camera and measure in situ electric field strengths; PGB and RPMP constructed the experimental setup; PGB and KS performed the measurements with live animals and collected the data; PGB and KS performed the in situ field strength measurements; MJL performed the simulations of the electric field; PGB, AH, and KS performed the statistical analyses; all authors interpreted the data and discussed the results; PGB drafted the initial manuscript and figures, with contributions by AH, KS, RPMP, and MJL; all authors contributed to the critical revision of the manuscript and figures, and approved the final version.

4.5.3 Funding

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Supplementary material

Supplementary material 4.1. Feeding of experimental animals.
Supplementary material 4.2. *In situ* electric field measurements in the experimental setup.
Supplementary material 4.3. Body length measurements.
Supplementary material 4.4. Response curves, receiver-operating characteristic (ROC) curves, and cut-off plots for all specimens per species.
Supplementary material 4.5. Individual-specific area under the curve (AUC) values of the receiver-operating characteristic (ROC) curves.

Supplementary material 4.1. Feeding of experimental animals.

Small-spotted catshark (*Scyliorhinus canicula*) and thornback ray (*Raja clavata*) were fed with live king ragworm (*Alitta virens*) and whole, defrosted raw brown shrimp (*Crangon crangon*) during the first weeks of acclimatization to captive housing conditions. Thereafter, small-spotted catshark and thornback ray were fed with a mix of cut, defrosted Atlantic cod (*Gadus morhua*), whiting (*Merlangius merlangus*), tub gurnard (*Chelidonichthys lucerna*), grey gurnard (*Eutrigla gurnardus*), and Atlantic mackerel (*Scomber scombrus*). Turbot (*Scophthalmus maximus*) was mainly fed with 9 mm pellets (Repro, Alltech Coppens) and, at most once a week, with the aforementioned mix of cut, defrosted fish species. European seabass (*Dicentrarchus labrax*) and common sole (*Solea solea*) were fed with 4.5 mm pellets (Supreme 15, Alltech Coppens). Any uneaten food was removed within one day after feeding.

Supplementary material 4.2. *In situ* electric field measurements in the experimental setup.

To verify the numerical simulation of the electric field with the AC/DC package in COMSOL Multiphysics (COMSOL Multiphysics®, n.d.), we measured the *in situ* electric field in our experimental setup. Hereto, we constructed a framework over the experimental tank that enabled us to measure the electric field strength at different locations in the water. We used two custom-made measurement probes that were placed at the desired location in the experimental tank. The probes consisted of a fully insulated shaft and head, from which two brass rods (⌀1 mm x 0.5 cm length) protruded at 0.5 cm or 2 cm apart, measured from core to core (**Figure S4.2A**).

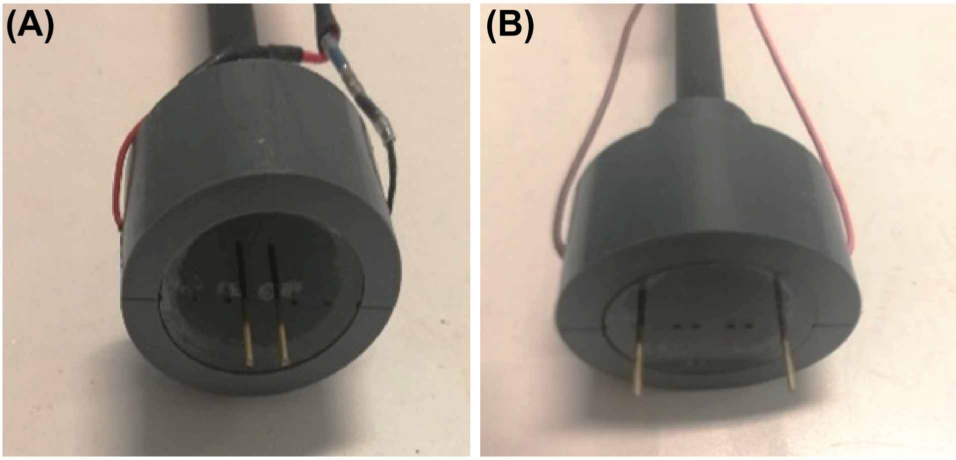

Figure S4.2A. Photographs of the probes that were used to measure the *in situ* electric field strength in the experimental setup. Brass rod probes were 0.5 cm long with a diameter of 1 mm and were spaced at **(A)** 0.5 cm and **(B)** 2 cm.

Each brass rod was connected to an insulated copper wire, which were connected to a SCB-68A interface, which was, in turn, connected via a SHC68-68-EPM 2 m cable to a National Instruments PCI-6221 data acquisition card (VHDCI front connection type). This card was read out using custom-made software programmed in `Python` (Python Software Foundation, n.d.). The algorithm computed the mean electric field strength (V_{pk}) for all positive and negative (absolute values) pulses combined and the respective standard deviation. In contrast, the numerical simulation in COMSOL Multiphysics computed the electric field strength based on one pulse of 10 V ($V_{pk\text{–}pk}$). Hence, data of a 10 V stimulation during the *in situ* measurements can be compared directly with the numerical simulation. Due to the technical properties of our pulse generator equipment and technical specification of our measurement equipment, we could only reliably measure the electric field strengths for pulse amplitudes up to 10 V. Our pulse generator generated a ‘floating’ electric field. Each electrode was a tri-state connection, meaning that unselected, it was in floating mode (via 560 kΩ to the anode of the connected power supply with a current leak of maximum 0.1 mA). We measured the electric field strengths differentially between the two brass rods, because for a single-ended measurement one would need to connect one line to a ground, creating a short circuit. For the differential measurement, we used the internal reference of the data acquisition card. However, this range was limited, meaning that if we increased the pulse amplitude beyond 10 V (i.e. $V_{pk\text{–}pk}$ of 20 V), the signal could float away, resulting in unreliable data.

We performed *in situ* measurements by quantifying the electric field strength (which is a vector quantity; i.e. having both a magnitude and direction) in two, orthogonal directions by rotating the measurement probe 90 degrees at the same location. Hereafter, we computed the absolute electric field strength using Pythagoras’ theorem. An important difference between the numerical simulation and *in situ* data is that for the latter we determine the electric field strength over a certain distance (i.e. the probe distance of 0.5 or 2 cm) whilst these are point values from the simulation. This may lead to deviations when comparing these data (see below). Another deviation may be caused because, for the *in situ* data, the potential difference is measured from the surface-to-surface of the probe, whilst we calculate the electric field strength based on the distance of the probes' cores. Finally, the presence of non-conductive probe parts may slightly deform the electric field, which could also result in a deviation of the field strength values with respect to the simulation data.

With the electric field measurement setup, we determined the electric field strength as function of horizontal distance from an active electrode towards the centre of experimental setup, within the plane between the active electrode pair at a depth of 12.5 cm (**Figure S4.2B**). We measured the electric field strength at eleven locations. We compared these data with the numerical simulation data on the same coordinates to verify if the field has the same shape and quantity. The electric field shape is the same for the *in situ* and simulation data, showing an inverse-square law relationship. The electric field strengths are similar up to about 10 cm from the electrode, but deviation increases between the simulation and *in situ* measurement at closer distances. This difference is likely the result by the aforementioned causes because it only occurs at close range, where these effects are expected to be larger. Because the electric field strength between the *in situ* measurements and numerical simulation are similar, we assumed that using the latter dataset is justified without, e.g., any correction factor.

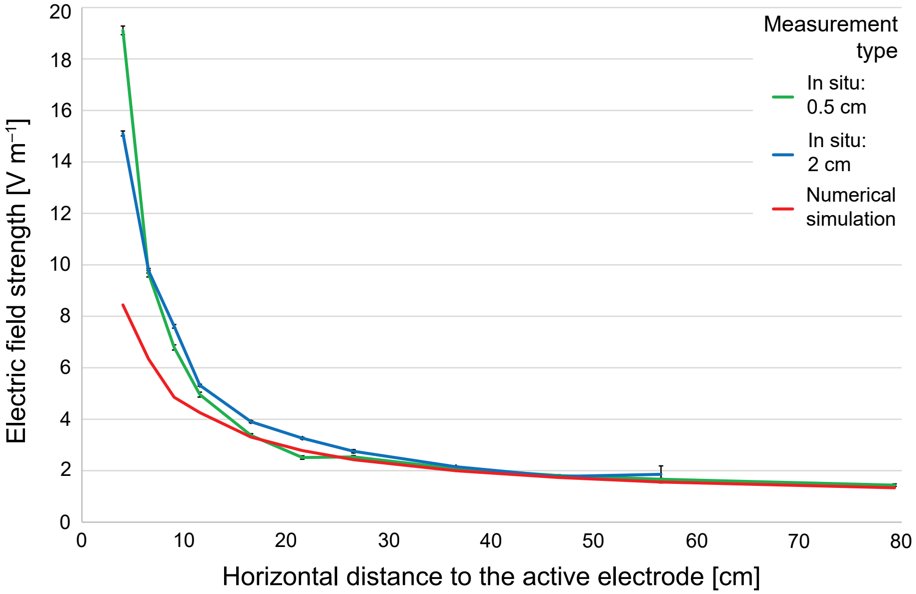

Figure S4.2B. Electric field strength as function of horizontal distance (measured at eleven locations) to the active electrode for the *in situ* electric field measurements (with the 0.5 cm and 2 cm measurement probes) and the numerical simulation data. Measurements and simulation data are from in the plane between the active electrode pair at a depth from the bottom upwards of 12.5 cm and 10 cm respectively. Error bars indicate the standard deviation of the measurement.

Furthermore, we quantified electric field strength as function of pulse amplitude on the electrode pair for different distances with respect to the nearest electrode (**Figure S4.2C**). Hereto, we varied pulse amplitude on the active electrodes (5, 7.5, and 10 V) to quantify the relationship of electric field strength and pulse amplitude, which was linear. Therefore, we linearly scaled the field strengths with the simulated field strengths at 10 V.

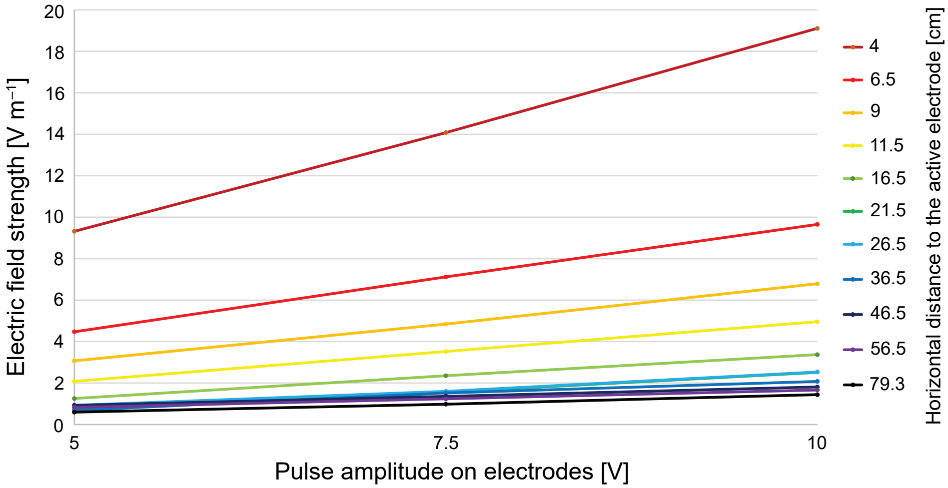

Figure S4.2C. Electric field strength as function of pulse amplitude on the electrode pair measured with the 0.5 cm probe at eleven locations in the same plane and depth from the active electrode (denoted by the colours). Electric field strength and pulse amplitude are linearly related.

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Supplementary material 4.3. Body length measurements

Table S4.3. Body length per individual per species. Total length was measured for small-spotted catshark and thornback ray. Standard length was measured for European seabass, turbot, and common sole.

Species	**Specimen number**	**Body length [cm]**
Small-spotted catshark (*Scyliorhinus canicula*)	1	53.7
	2	57.3
	3	56.2
	4	59.1
	5	58.6
	6	64.7
Thornback ray (*Raja clavata*)	1	50.7
	2	33.8
	3	52.8
	4	57.7
	5	57.5
	6	53.3
European seabass (*Dicentrarchus labrax*)	1	27.0
	2	29.8
	3	28.7
	4	30.9
	5	31.0
	6	31.2
	7	30.6
	8	29.5
	9	29.0
Turbot (*Scophthalmus maximus*)	1	23.4
	2	24.8
	3	30.7
	4	26.6
	5	30.5
Common sole (*Solea solea*)	1	30.3
	2	35.7
	3	31.0
	4	29.8
	5	25.8
	6	28.7
	7	26.6

Supplementary material 4.4. Response curves, receiver-operating characteristic (ROC) curves, and cut-off plots for all specimens per species.

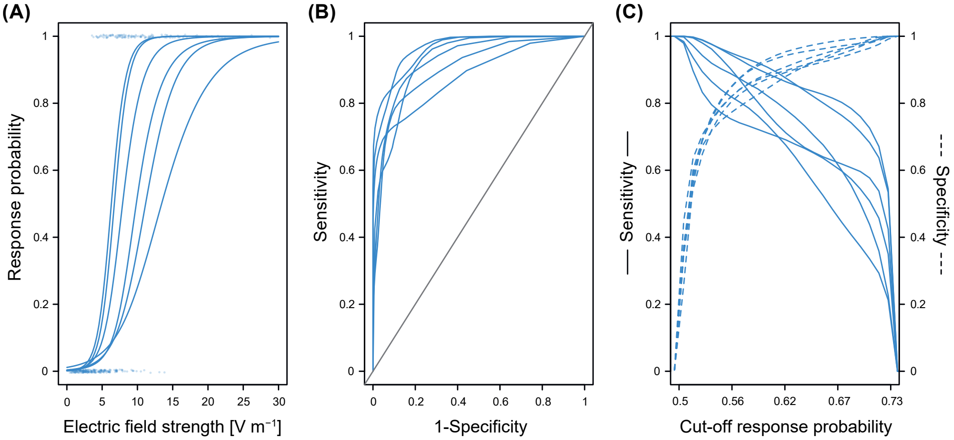


Figure S4.4A. Individual responses to the electric stimulus and corresponding receiver-operating characteristic (ROC) analysis for all six small-spotted catshark (*Scyliorhinus canicula*) specimens. **(A)** Response probability as a function of electric field strength (individual response curve). Dots at the bottom and top indicate the raw data for absence or presence of a response respectively. Note that some raw response values are not shown, because these are out of range to increase details of the response curves. **(B)** ROC curve or true positive rate (sensitivity) as a function of false negative rate (1-specificity). **(C)** True positive rate (sensitivity) and true negative rate (specificity) as a function of various cut-off response probabilities. The optimal cut-off response probability was defined as the cut-off with the highest Youden Index, specifying the highest sum of sensitivity and specificity (not shown).

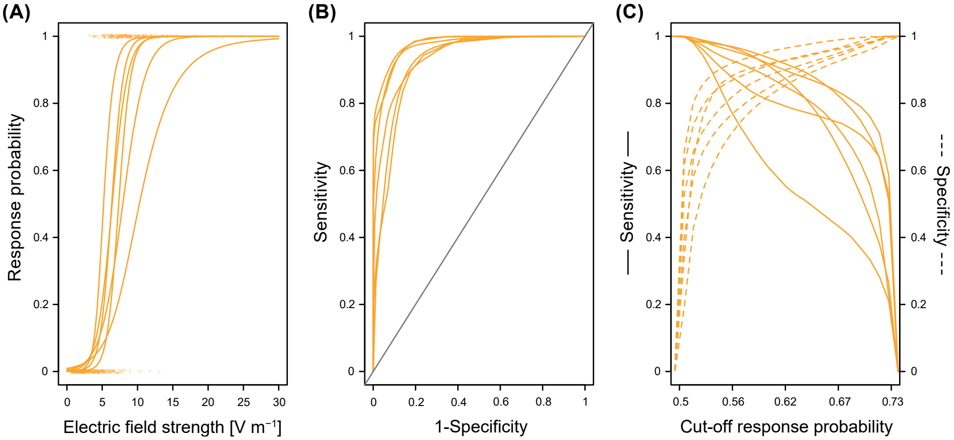


Figure S4.4B. Individual responses to the electric stimulus and corresponding receiver-operating characteristic (ROC) analysis for all six thornback ray (*Raja clavata*) specimens. **(A)** Response probability as a function of electric field strength (individual response curve). Dots at the bottom and top indicate the raw data for absence or presence of a response respectively. Note that some raw response values are not shown, because these are out of range to increase details of the response curves. **(B)** ROC curve or true positive rate (sensitivity) as a function of false negative rate (1-specificity). **(C)** True positive rate (sensitivity) and true negative rate (specificity) as a function of various cut-off response probabilities. The optimal cut-off response probability was defined as the cut-off with the highest Youden Index, specifying the highest sum of sensitivity and specificity (not shown).

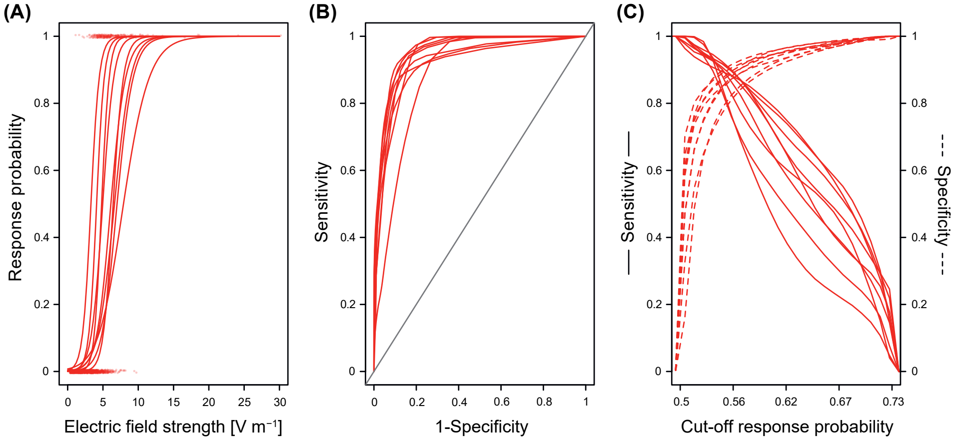


Figure S4.4C. Individual responses to the electric stimulus and corresponding receiver-operating characteristic (ROC) analysis for all nine European seabass (*Dicentrarchus labrax*) specimens. **(A)** Response probability as a function of electric field strength (individual response curve). Dots at the bottom and top indicate the raw data for absence or presence of a response respectively. Note that some raw response values are not shown, because these are out of range to increase details of the response curves. **(B)** ROC curve or true positive rate (sensitivity) as a function of false negative rate (1-specificity). **(C)** True positive rate (sensitivity) and true negative rate (specificity) as a function of various cut-off response probabilities. The optimal cut-off response probability was defined as the cut-off with the highest Youden Index, specifying the highest sum of sensitivity and specificity (not shown).

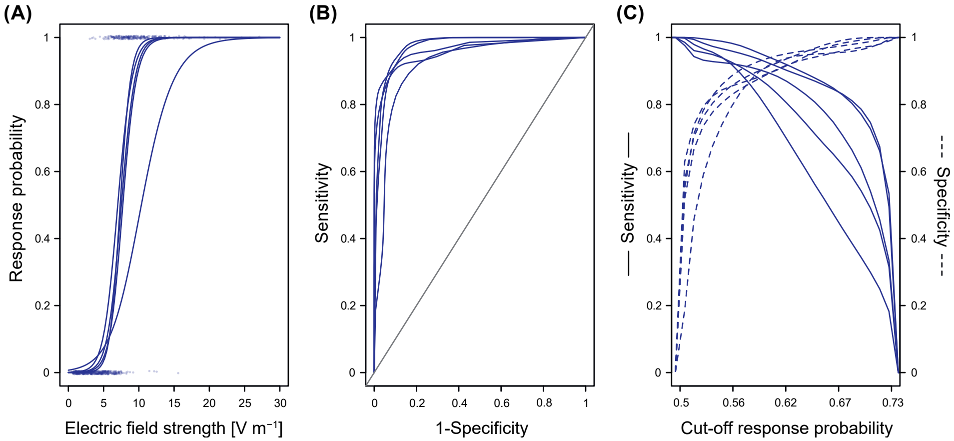


Figure S4.4D. Individual responses to the electric stimulus and corresponding receiver-operating characteristic (ROC) analysis for all five turbot (*Scophthalmus maximus*) specimens. **(A)** Response probability as a function of electric field strength (individual response curve). Dots at the bottom and top indicate the raw data for absence or presence of a response respectively. Note that some raw response values are not shown, because these are out of range to increase details of the response curves. **(B)** ROC curve or true positive rate (sensitivity) as a function of false negative rate (1-specificity). **(C)** True positive rate (sensitivity) and true negative rate (specificity) as a function of various cut-off response probabilities. The optimal cut-off response probability was defined as the cut-off with the highest Youden Index, specifying the highest sum of sensitivity and specificity (not shown).

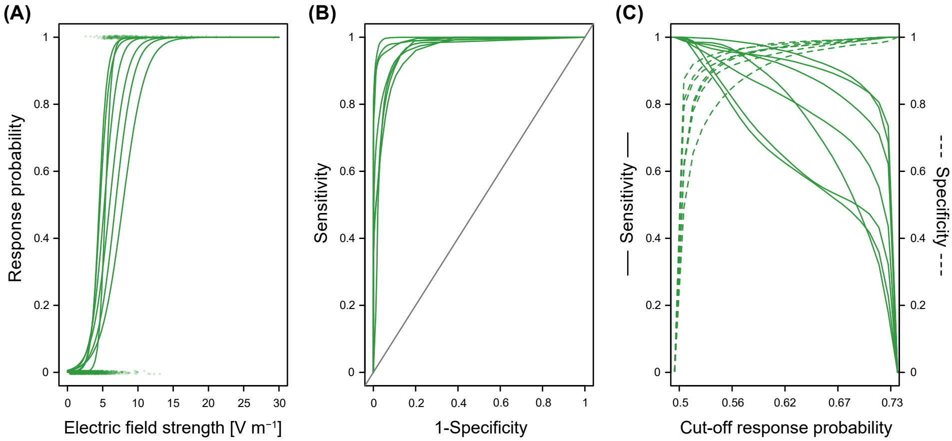
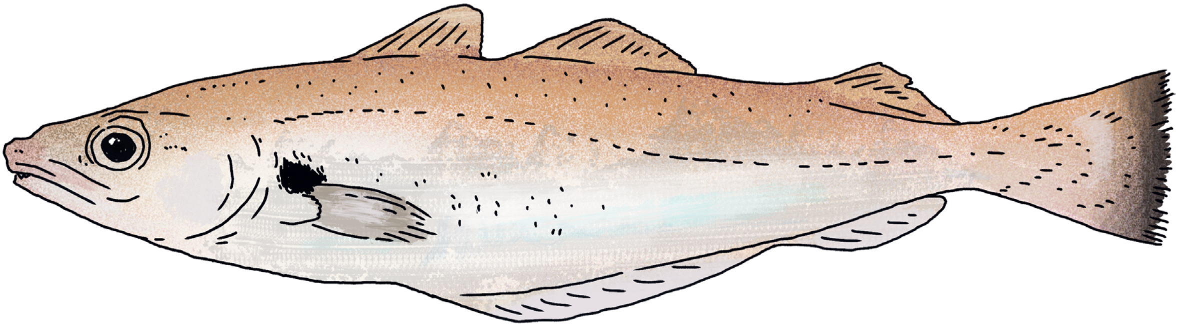


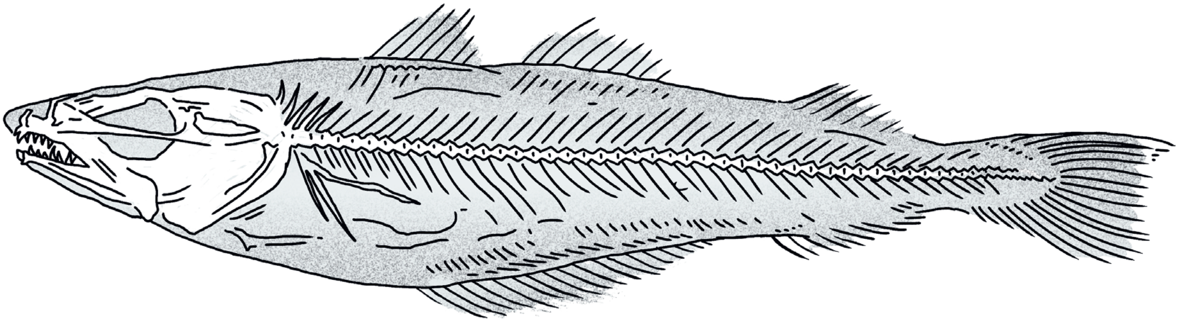
Figure S4.4E. Individual responses to the electric stimulus and corresponding receiver-operating characteristic (ROC) analysis for all seven common sole (*Solea solea*) specimens. **(A)** Response probability as a function of electric field strength (individual response curve). Dots at the bottom and top indicate the raw data for absence or presence of a response respectively. Note that some raw response values are not shown, because these are out of range to increase details of the response curves. **(B)** ROC curve or true positive rate (sensitivity) as a function of false negative rate (1-specificity). **(C)** True positive rate (sensitivity) and true negative rate (specificity) as a function of various cut-off response probabilities. The optimal cut-off response probability was defined as the cut-off with the highest Youden Index, specifying the highest sum of sensitivity and specificity (not shown).

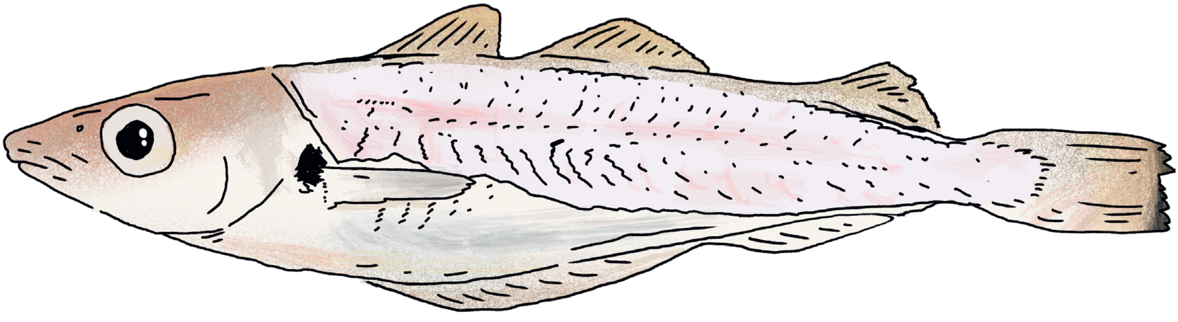
Supplementary material 4.5. Individual-specific area under the curve (AUC) values of the receiver-operating characteristic (ROC) curves.

Table S4.5. Individual-specific area under the curve (AUC) values of the receiver-operating characteristic (ROC) curves presented in **Supplementary material 4.4**.

Species	Specimen number	AUC value [–]
Small-spotted catshark (*Scyliorhinus canicula*)	1	0.942
	2	0.895
	3	0.911
	4	0.936
	5	0.966
	6	0.967
Thornback ray (*Raja clavata*)	1	0.971
	2	0.980
	3	0.945
	4	0.929
	5	0.941
	6	0.982
European seabass (*Dicentrarchus labrax*)	1	0.929
	2	0.925
	3	0.946
	4	0.944
	5	0.892
	6	0.963
	7	0.954
	8	0.960
	9	0.930
Turbot (*Scophthalmus maximus*)	1	0.958
	2	0.926
	3	0.957
	4	0.982
	5	0.975
Common sole (*Solea solea*)	1	0.971
	2	0.956
	3	0.992
	4	0.989
	5	0.969
	6	1.000
	7	0.960







Chapter 5

Internal injuries in whiting (*Merlangius merlangus*) caught by tickler-chain and pulse-trawl gears

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Abstract

Electrical pulse fishing has been widely adopted by Dutch fishermen as an economically viable alternative to tickler-chain trawling for common sole (*Solea solea*) in the North Sea. A major concern about pulse trawling is that it may cause a high incidence of spinal injuries, as previously shown for Atlantic cod (*Gadus morhua*). To find out whether other gadoids are similarly affected, we studied injury occurrences in whiting (*Merlangius merlangus*) catches of commercial vessels. To distinguish mechanically and electrically-induced injuries, we compared injuries for pulse gears with electrical pulses either turned on or off and we compared injuries from pulse-trawl catches with those in tickler-chain beam trawling. We visualised spinal injuries with X-radiography, followed by dissection to reveal internal haemorrhages. Both injury types were categorised on a severity scale and their location was quantified along the anteroposterior fish axis. In modelling the effect of catch method, fish-length and fishing-trip effects were taken into account. Spinal injury probabilities in pulses-on and pulses-off catches were low (on average ≤3%) and no evidence for electrically-induced injuries was found. Severe spinal injury probability was slightly higher in tickler-chain catches (2.5%) than in pulses-on samples (0.8%) and this difference increased for smaller specimens. The locations of spinal injuries did not show a consistent pattern as previously shown in Atlantic cod exposed to electrical pulses in laboratory conditions. Severe haemorrhage probabilities were also low, but slightly higher in the pulses-on samples (1.8%) compared to fish caught with tickler chains (0.3%), especially for the larger specimens. The locations of severe haemorrhages in pulses-on catches, and a correlation with spinal injury occurrences, suggest that they may be (partly) related to electrical-pulse exposure. Overall, our results indicate that spinal injuries in whiting are rare and primarily due to mechanical impact. Severe haemorrhages may be partially related to electrical pulsing but incidences are low and coincide with a significantly lower chance for spinal injuries. These findings suggest that pulse trawling is unlikely to impose increased mortality on whiting populations compared to the tickler-chain technique.

Keywords: bottom trawling; electrical pulse fishing; haemorrhage; spinal injury; North Sea

5.1 Introduction

Towed demersal trawls are used globally in marine capture fisheries (Watson *et al.*, 2006; Hiddink *et al.*, 2017; Amoroso *et al.*, 2018; Cashion *et al.*, 2018; Watson and Tidd, 2018; Zeller *et al.*, 2018). In conventional beam trawling, tickler chains, chain mats, or bobbins are used to mechanically stimulate the target species from the seabed to be caught in the following nets (Jennings and Kaiser, 1998; Rijnsdorp *et al.*, 2008; Soetaert *et al.*, 2015a; Eigaard *et al.*, 2016). Beam trawling is commonly criticised for its negative effects on the environment (e.g., de Groot, 1984; Jones, 1992; Lindeboom and de Groot, 1998; Paschen *et al.*, 2000; Catchpole *et al.*, 2008; Poos *et al.*, 2013; Uhlmann *et al.*, 2014; Bayse *et al.*, 2016; McConnaughey *et al.*, 2020). Electrical pulse trawling is a promising alternative to the beam trawl fishery targeting common sole (*Solea solea*) in the southern North Sea (Soetaert *et al.*, 2015a, 2019). This so-called pulse trawl gear is installed on the same vessel type as the tickler-chain gear (Soetaert *et al.*, 2015a; Poos *et al.*, 2020), where the chains are replaced by electrode arrays to generate electric fields (Soetaert *et al.*, 2019). These pulsed electric fields chase common sole from the seabed's sediment and induce involuntary muscle contractions which immobilises the fish in front of the netting, enabling subsequent capture (de Groot and Boonstra, 1970; Stewart, 1977; Soetaert *et al.*, 2015a, 2019).

Compared to tickler chains, the pulse fishing technique has several advantages, including lower fuel consumption (van Marlen *et al.*, 2014; Poos *et al.*, 2020), reduced seabed disturbance (Depestele *et al.*, 2016, 2019; Tiano *et al.*, 2019; Rijnsdorp *et al.*, 2020, 2021; De Borger *et al.*, 2021), and reduced impact on benthic invertebrates (Soetaert *et al.*, 2015b, 2016a; Bergman and Meesters, 2020; Boute *et al.*, 2021) although habitat-dependent (Tiano *et al.*, 2020), as well as increased selectivity (van Marlen *et al.*, 2014; Poos *et al.*, 2020), higher discard survival (van der Reijden *et al.*, 2017), and higher revenues (Batsleer *et al.*, 2016). However, concerns have been expressed regarding the occurrence of internal injuries in non-target fish species exposed to the pulsed electric field (van Marlen *et al.*, 2014; de Haan *et al.*, 2016; Desender *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c; ICES, 2018; Quirijns *et al.*, 2018).

Fishery methods may cause internal injuries in fish during the catch process (Suuronen, 2005; Cook *et al.*, 2019), for example, by imposing an external mechanical load on the body. In beam trawling, this can be caused by gear components and handling on deck, and can be affected by the towing speed, catch composition, and catch volume (ICES, 2018). In pulse trawling, besides mechanically-induced trauma, internal injuries may also be caused by excessive muscle contractions induced by electrical stimulation. Pulse fishing for common sole also exposes non-target fishes to high electric field strengths in close proximity to the electrodes, which are dragged over the seafloor (de Haan *et al.*, 2016; de Haan and Burggraaf, 2018). Simultaneous induction of muscle contractions on both sides may lead to spinal injuries and haemorrhages (van Marlen *et al.*, 2014; de Haan *et al.*, 2016; Soetaert *et al.*, 2016c, 2016b, 2019). For large fish, this may be irrelevant as they are retained, killed, and subsequently processed on board, but for small specimens, internal injuries may reduce the chance for escaping from the netting, or may increase the risk for predation if not retained in the netting. Internal injuries may thus lead to increased fishing mortality (e.g., Chopin and Arimoto, 1995; Kaiser and Spencer, 1995; Ryer, 2004; Broadhurst *et al.*, 2006; Gilman *et al.*, 2013; Raby *et al.*, 2014) and, in turn, lead to disturbed food web structures which subsequently affect population dynamics of other species (Kaiser *et al.*, 2002; Hiddink *et al.*, 2011; van Denderen *et al.*, 2013; Collie *et al.*, 2017). To quantify potential negative impacts of pulse trawling on population dynamics and the ecosystem, it is essential to assess internal injuries resulting from different capture methods, especially in small specimens.

The susceptibility to electrical-pulse-induced internal injuries in sole-targeting marine electrotrawling has been studied in only few fish species. Common sole and European seabass (*Dicentrarchus labrax*) were found insensitive to electrical-pulse induced internal injuries (Soetaert *et al.*, 2016b, 2018). In Atlantic cod, high incidences (7–11%) of spinal injuries and haemorrhages have been reported for fish caught with pulse gears or electrified benthos release panels (van Marlen *et al.*, 2014; Soetaert *et al.*, 2016d). Laboratory experiments confirmed that these injuries likely resulted from electrical stimulation (de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c), although injury incidences were variable (0–37%; depending on fish size and electric field strength). It remained unclear, however, whether high incidence rates are specific for Atlantic cod, or also found in other Gadidae. Whiting is an important exploited gadoid (Greater North Sea ecoregion single-stock catch advice in 2021 of 26,304 metric tonnes) that is frequently caught by bottom trawlers targeting common sole and a key species of the North Sea demersal fish assemblages (Greenstreet *et al.*, 1999; Quirijns and Pastoors, 2014; Heessen *et al.*, 2015; Lynam *et al.*, 2017; ICES, 2020a, 2020b). High incidences of pulse-induced injuries in whiting may, therefore, have a large ecological effect. However, except for a single injured specimen found in 57 filleted fish in a field study (van Marlen *et al.*, 2014), no information on injury incidences in whiting during pulse fishing is available. To fill this gap, we extensively sampled whiting catches on board commercial pulse and tickler-chain vessels and quantified internal injuries.

To assess incidences of internal injuries in whiting and determine whether injuries are related to electrical pulsing, we performed two comparisons. First, we compared injuries in whiting sampled from pulse trawlers catches with the electrical pulses either turned on or off (experiment 1). By comparing the injury incidences in these catches, we pinpoint whether whiting is vulnerable to muscle-contraction induced injuries. Secondly, we compare whiting catches between pulse trawlers with pulses-on and conventional tickler-chain trawlers (experiment 2). For the ecological interpretation, the presence of injuries in small specimens that could escape the nets before the end of the tow, is especially relevant. To increase catchability of small whiting, we used cover nets during a number of fishing trips. After landing, sampled whiting were X-radiographed to visualise spinal injuries, followed by filleting dissection to reveal internal haemorrhages.

In Atlantic cod, electrical-pulse-induced injuries are typically located in the anterior part of the caudal region and can be accompanied by large haemorrhages (de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c). To reveal whether whiting show a similar pattern, we assessed the correlation between haemorrhages and spinal injuries, modelled the effect of haemorrhage probability as function of catch method and fish length, and mapped injury locations on the anteroposterior axis of the fish. Together with a quantitative comparison of spinal injury incidences between pulses-on and pulses-off catches (experiment 1) and between pulses-on and tickler chains (experiment 2), these analyses will show whether internal injuries due to electrical pulsing are also found in whiting. More in general, these findings will contribute to understanding the effects of beam trawling and pulse trawling on the ecosystem.

5.2 Materials and methods

5.2.1 Collection of animals

Whiting (*Merlangius merlangus*) were randomly sampled from catches on board commercial vessels targeting common sole (*Solea solea*) by scientists from Wageningen Marine Research and the Experimental Zoology Group (EZO) of Wageningen University & Research (**Table 5.1** and **5.2**). In the case of V8 and V9, whiting were sampled directly after landing, as many whiting discards were retained during the fishing trip in the presence of an independent observer. Fishing trips lasted five days, from Sunday evening to Friday morning. Except for the 3–9 hours required to reach the fishing grounds, fishing continued day-and-night in hauls lasting about two hours. After a haul, fish were unloaded in hoppers on deck, and were sorted and gutted while the next haul started immediately. Sampling occurred during as many hauls as possible, when the fish were on the conveyor belt, before gutting by the fishermen. Pulse trawlers used a PulseWing from HKF Engineering with a Pulsed Bipolar Current waveform (Soetaert *et al.*, 2019) and tickler-chain beam trawlers used a SumWing. All vessels used the same

Table 5.1. Experiment 1 trawler sampling overview per vessel (anonymised coding) and trip with catch year and week, treatment, number of collected animals per net part, towing speed, and electrical-pulse settings.

Vessel	**Year**	**Week**	**Treatment**	**Number of animals**		**Towing speed (mean ± SD) [kn]**	**Electrical pulse settings (mean ± SD)**			
				Cod-end	**Cover**		**Amplitude [V_{pk}]**	**Frequency [Hz]**	**Width [µs]**	**Duty cycle [–]**
V1	2016	29	Pulses on	48	333	5.1 ± 0.1	54.3 ± 2.9	30 ± 0	349.3 ± 2.5	2.1%
			Pulses off	67	198		NA			
V2	2018	4	Pulses on	40	NA	5.0 ± 0.1	54.3 ± 3.1	30 ± 0	300 ± 0	1.8%
			Pulses off	186			NA			
V3	2018	8	Pulses on	68		5.0 ± 0.1	56.1 ± 2.9	22.5 ± 0	390 ± 0	1.8%
			Pulses off	135			NA			

Table 5.2. Experiment 2 trawler sampling overview per vessel (anonymised coding) and trip with catch year and week, treatment, number of collected animals per net part, towing speed, and electrical-pulse settings.

Vessel	Year	Week	Treatment	Number of animals		Towing speed (mean ± SD) [kn]	Electrical pulse settings (mean ± SD)			
				Cod-end	Cover		Amplitude [V_{pk}]	Frequency [Hz]	Width [µs]	Duty cycle [–]
V1	2016	29	Pulses on	48	333	5.1 ± 0.1	54.3 ± 2.9	30 ± 0	349.3 ± 2.5	2.1%
V2	2016	41		563	NA	5.0 ± 0.1	55.5 ± 3.0	30 ± 0	350 ± 0	2.1%
	2018	4		40		5.0 ± 0.1	54.3 ± 3.1	30 ± 0	300 ± 0	1.8%
V4	2017	6		125		4.7 ± 0.1	58.7 ± 3.0	30 ± 0	300 ± 0	1.8%
V5	2017	7		85		4.9 ± 0.1	55.3 ± 2.9	30 ± 0	350 ± 0	2.1%
V6	2017	24		0	609	4.9 ± 0.3	58.3 ± 2.9	30 ± 0	350 ± 0	2.1%
		33		0	275	4.9 ± 0.3	58.3 ± 3.1	30 ± 0	350 ± 0	2.1%
V7	2017	24		6	NA	5.0 ± 0.1	56.0 ± 3.1	30 ± 0	335.3 ± 5.0	2.0%
		44		149		5.0 ± 0.1	55.5 ± 3.0	30 ± 0	330 ± 0	2.0%
V8	2018	4		198		5.0 ± 0.1	54.6 ± 3.1	30 ± 0	350 ± 0	2.1%
V9	2018	6		117		5.0 ± 0.1	54.8 ± 3.2	30 ± 0	330 ± 0	2.0%
V3	2018	8		68		5.0 ± 0.1	56.1 ± 2.9	22.5 ± 0	390 ± 0	1.8%
V10	2018	23	Tickler chains	93		6.1 ± 0.1	NA			
		26		9		6.1 ± 0.1				
V11	2018	47		795		6.5 ± 0.3				
	2019	9		251		6.5 ± 0.3				

towing speed as they would use without sampling, which was typically lower for pulse gear than tickler-chain gears (van Marlen *et al.*, 2014; Depestele *et al.*, 2019; Poos *et al.*, 2020; Rijnsdorp *et al.*, 2020). Electrical-pulse settings were highly similar between trips and vessels and matched those of 33 pulse trawlers in the fleet using the HFK system (ICES, 2020c). Fish samples were stored in sealed plastic bags on ice below 4 °C. After landing, all fish were stored at –20 °C in the freezer facilities of EZO in Wageningen.

In experiment 1, specimens were collected during three fishing trips of different pulse trawlers with electrical stimulation turned on (**Table 5.1**). Two vessels fished for one tow with the pulses turned off on both starboard and portside, from which all whiting were collected. For the other tows with pulses on, whiting were randomly sampled, to obtain similar numbers of fish. The third vessel fished with the pulses turned off, either on starboard or portside, for three tows and whiting were randomly sampled from the catch with pulses on and with pulses off from the same hauls. All three vessels used 80 mm diamond-shaped mesh cod-ends. To include small fish that might have escaped from the cod-end, one vessel was equipped with 40 mm mesh cover nets that spaciously fitted over the cod-end. Specimens were sampled from both the cod-ends and cover nets, for both treatments.

In experiment 2, specimens were collected from nine pulse trawlers during twelve fishing trips (including the pulses-on specimens from experiment 1) and from two conventional beam trawlers using tickler chains during four fishing trips (**Table 5.2**). Similar to experiment 1, all vessels used 80 mm diamond-shaped mesh cod-ends. Pulse trawler V6 fished with trouser nets equipped with 40 mm mesh covers from where the specimens were sampled. The V10 and V11 used conventional tickler-chain beam trawls with 8 and 6 shoe-tickler chains and 12 and 14 net-tickler chains respectively.

Collection of fish was approved by the Animal Welfare Body of Wageningen University, the Animal Ethics Committee of Wageningen University & Research, and the Central Authority for Scientific Procedures on Animals (application number AVD1040020184945). Whiting has a legal European Union (EU) regulated minimal landing size of 27 cm (total length) in the North Sea (Council of the European Union, 1998; European Parliament and Council, 2019). In case collecting undersized specimens could not occur under the Landing Obligation as part of the EU Common Fisheries Policy due to phased implementation (European Parliament and Council, 2013; European Commission, 2018, 2019), collection occurred with permission of the authorities via derogations granted by the Netherlands Enterprise Agency. The use of cover nets for experimental purposes was approved by the Netherlands Enterprise Agency.

5.2.2 X-ray and filleting dissection analysis

To visualise internal injuries, we defrosted the fish and subsequently X-rayed them both laterally and dorsoventrally using a Philips SRM 0310 X-ray tube (serial number 923436) with a 46401G housing. The X-rays were generated with a Philips Super CP 80 (serial number 953031) and emitted from a Philips Optimus M200 holder through a Philips XD6028 collimator using a Philips Super CP 50 control panel. Total filtration of the X-ray tube was 2.3 mm Al and total filtration by the collimator was 0.2 mm Al. Depending on fish size, X-radiographs were shot with settings in the range of 40–71 kV and 32–71 mAs and captured by either a 35.2 x 42.8 cm standard plate (4020 x 4892 px, pixel size 87.5 µm, 12 bit) or a 23.8 x 29.7 cm mammography plate (5440 x 6776 px, 43.75 µm, 12bit). For processing speed and efficiency, multiple fish were X-radiographed simultaneously per plate. Images were read out with a Regius model 110HQ digitizer from Konica Minolta. Distance between X-ray source and plate was 127 cm. After X-radiography, fish standard length was measured to the nearest millimetre (Rabone Chesterman No 47R mounted on a measuring board), followed by dissection to expose internal haemorrhages. For dissection, each fish was filleted on the left and right side, and photographed with a Nikon D700 digital camera with a 24–120mm f/3.5-5.6G ED-IF AF-S VR NIKKOR lens. Specimens collected from V1 were only X-rayed laterally and not inspected for internal haemorrhages.

5.2.3 Injury category and scoring system

To enable standardised and consistent scoring, internal injuries were categorised based on (i) own observations during an exploratory assessment, (ii) injuries scored in freshwater electrofishing studies (Fredenberg, 1992; Hollender and Carline, 1994; Dalbey *et al.*, 1996; Snyder, 2003), and (iii) those reported in laboratory exposure experiments in the context of marine electrotrawling (de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c). Spinal injuries were scored in three categories: minor, moderate, and severe (**Figure 5.1**). Minor spinal injuries were deformations of one or multiple vertebrae including minor subluxation. Moderate spinal injuries included a subluxation or compression of several vertebrae (i.e., spinal misalignment) with minor fractures only. Severe spinal injuries were fractured and/or dislocated vertebrae, where the spinal column was either slightly or completely displaced. Other skeletal deformities that were obviously unrelated to acute injuries, such as the presence of additional spines, spinal curvature linked to developmental luxation, and block vertebrae, as also seen in other bony fish species, were not taken into account (Ford and Bull, 1926; McCrimmon and Bidgood, 1965; Gill and Fisk, 1966; Slooff, 1982; Sharber and Carothers, 1988; Fredenberg, 1992; Thompson *et*

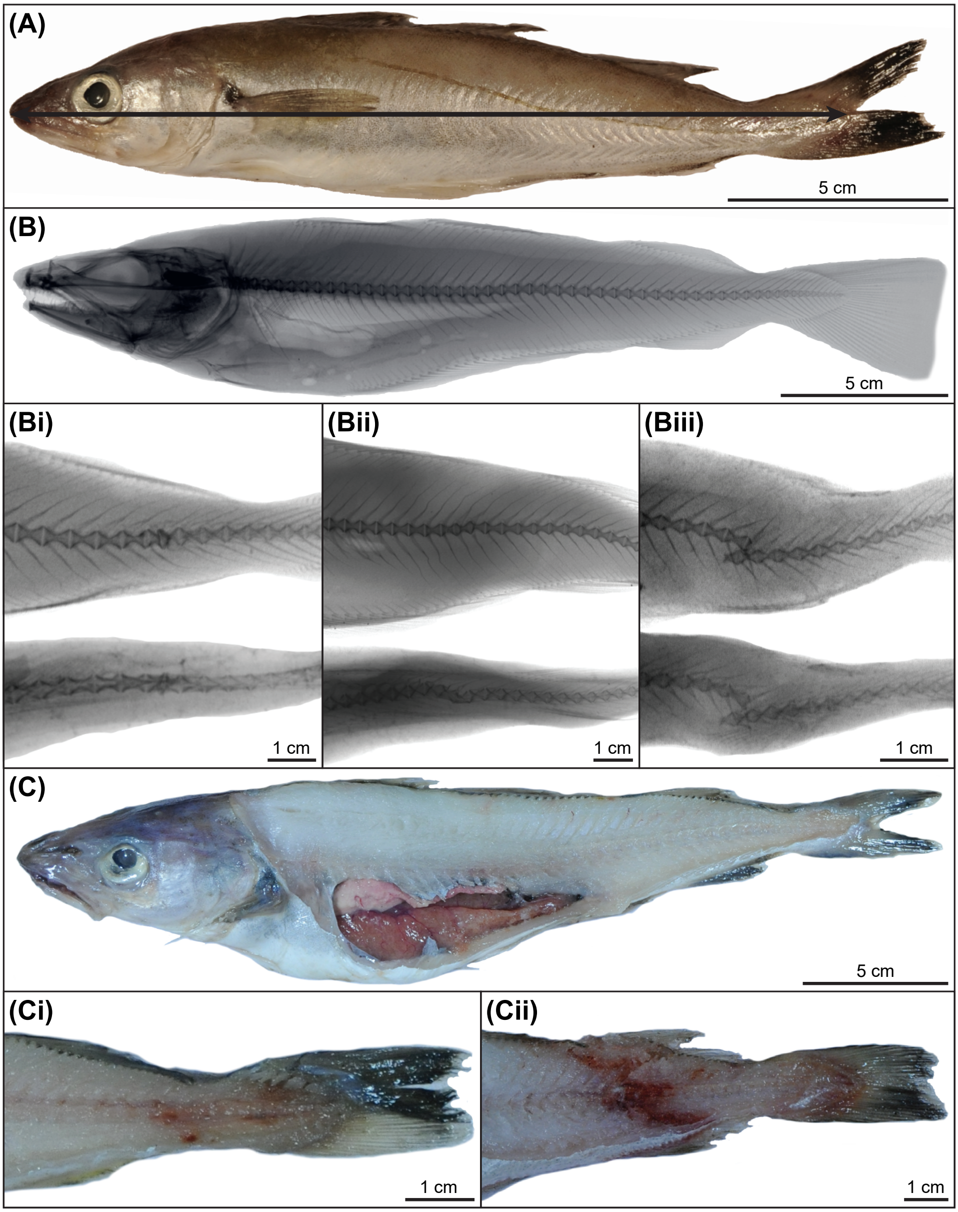

Figure 5.1. Categorisation of internal injury types. **(A)** The location of internal injuries was quantified on the anteroposterior axis of the fish relative to the snout and caudal fin as indicated by the black double arrow. **(B)** Lateral X-radiograph of a whiting without injury. Spinal injuries were subdivided into **(Bi)** minor, **(Bii)** moderate, and **(Biii)** severe (top and bottom images are lateral and dorsoventral X-radiographs of the same fish respectively). **(C)** Photograph of a filleted, uninjured whiting. Haemorrhages were subdivided into **(Ci)** minor and **(Cii)** severe.

al., 1997; Kranenbarg *et al.*, 2005; Fjelldal *et al.*, 2009, 2012, 2018; Opstad *et al.*, 2013; Soetaert *et al.*, 2016b, 2016c, 2018). Internal haemorrhages were categorised as minor or severe (**Figure 5.1**). Haemorrhages caused by filleting could be distinguished from older haemorrhages as the blood from the former could be wiped away whereas blood from the latter was fixed in the flesh. Blood visible in the haemal canal running through the haemal arches of the spinal column could often be seen as a reddish smudge and was not scored. To score the presence and location of spinal injuries and haemorrhages on the anteroposterior axis of the fish, we used a custom-made software database system in `Python` (Python Software Foundation, n.d.), in combination with `OpenCV`, and `SQLiteManager` in Mozilla Firefox. The database system coupled all X-radiographs and dissection photographs to the relevant metadata, and recorded user input indicating the tip of the snout, base of the caudal fin (posterior end of the mid-lateral portion of the hypural plate) as well as location and severities of injuries.

5.2.4 Data analyses

To compare spinal injury incidences between catch methods we needed to take fish-length differences and unequal sample sizes between fishing trips into account. Hereto, we subdivided the fish caught per trip in 1 cm length classes (rounded down) and calculated the number of spinal injury observations as proportion of injuries per trip and per length class. We subsequently modelled the spinal injury incidence using generalized additive models (Wood, 2017), with the number of observations per trip and length class combination used as weight factor. Generalized additive models allow for non-linear dependencies of incidences on fish length (i.e., smooths), as found in laboratory-exposed Atlantic cod (de Haan *et al.*, 2016). To assess spinal injury probability, we added covariables to the null model (i.e., intercept only model), namely the catch method and/or the smoother for standard length, and their interaction, by fitting generalised additive models with a logit link for the binomially-distributed response:

$$\text{(i)} \qquad \mathrm{P}_{i,j} \sim Bin\left(1, p_{i,j}\right), logit\left(p_{i,j}\right) = \alpha + \beta_1 \mathrm{C}_{i,j} + \beta_2 s\left(\mathrm{SL}_{i,j}\right) + \beta_3 \mathrm{C}_{i,j} \times s\left(\mathrm{SL}_{i,j}\right),$$

where $\mathrm{P}_{i,j}$ corresponds to the proportion of spinal injuries per size class (i) in sampled trip (j). α is the overall intercept, C is the catch method (pulses-on versus pulses-off; pulses-on versus tickler chains), s is the non-linear smooth function, and SL is the fish standard length. Models were applied to the datasets of experiment 1 and 2 and each spinal injury severity category separately. Subsequent model selection was based on the Akaike Information Criterion (AIC) (Akaike, 1973). The model with fewer predicting variables was selected when ΔAIC < 2 (Burnham and Anderson, 2002). For all models, we used a basis dimension *k* of four, which restricts the degrees of freedom of the smoother function and thus precludes overfitting. Selected models were used to report potential statistical differences. However, because we were interested in both the effect of catch method and fish length, we use the model with both covariates to predict and plot spinal injury probability with 95% confidence intervals. Spinal injury probability was predicted for the length range that had a minimum of ten specimens in each length class for at least one of the catch methods.

We assessed the independence of occurrence for spinal injuries and haemorrhages using 3x4 contingency tables and a two-tailed Fisher's exact test, for both catch methods separately. The most severe injury category present per injury type was used per fish, so that each specimen occurred only once per contingency table. We subsequently modelled haemorrhage incidence as function of catch method (pulses-on versus tickler chains) and standard length with generalized additive models, similarly as done with spinal injuries described above with model (i).

Finally, we quantified injury locations on the anteroposterior axis of the fish per injury type and severity for both catch methods. This allowed for a direct comparison to highly localised injuries in Atlantic cod exposed to electrical stimulation (de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c), and may reveal potentially different causes in whiting.

All statistical analyses were performed in `R` v3.6.1 (R Core Team, 2019): generalized additive models were fitted using the `mgcv` package (Wood, 2021). Model fitted values and confidence intervals were calculated using the `predict` function in the `car` package (Fox and Weisberg, 2019) and back-transformed to the response scale with the inverse logit.

5.3 Results

In the three trips for experiment 1, we collected 489 specimens for pulses-on and 586 for pulses-off. In sixteen fishing trips for experiment 2, we collected an additional 2,127 specimens from pulse trawler catches and 1,148 specimens from tickler-chain catches. In total, we processed 8,054 X-ray images and 7,408 photographs of filleted fish. We did not observe external discolouration marks on specimens with a spinal injury and/or haemorrhage.

5.3.1 Experiment 1: pulses-on versus pulses-off

Length-frequency distributions for pulses-on and pulses-off samples were right-skewed and symmetrical with a maximum at 18 cm and 19 cm respectively (**Figure 5.2A**, **B**). Minor spinal injury incidence was, averaged over the three fishing trips, 3% in the pulses-on and 2% in pulses-off samples. Severe spinal injury incidences were 0.8% and 0.9%, respectively (**Table 5.3**). The incidence of spinal injuries was similar in the codend and cover nets (**Supplementary material 5.1**).

When modelling the observed data of **Table 5.3** as described in **Table 5.4**, we find no significant effect of catch method for both minor and severe spinal injury probability and neither find fish-length effects (**Figure 5.2C–F**). In conclusion, the probability of a spinal injury in electrical pulse fishing was low, and there was no significant change when the pulses were switched off.

Table 5.3. Observed mean spinal injury probability per severity category of whiting samples caught by pulses trawlers with and without electrical stimulation (experiment 1) per vessel (here equal to fishing trip). No moderate spinal injuries were observed.

Vessel	Catch method	Number of animals	Spinal injury probability per severity (*n*)	
			Minor	Severe
V1	Pulses on	381	3.7% (14)	0%
	Pulses off	265	3.8% (10)	0%
V2	Pulses on	40	0%	2.5% (1)
	Pulses off	186	0.5% (1)	1.1% (2)
V3	Pulses on	68	1.5% (1)	4.4% (3)
	Pulses off	135	0.7% (1)	2.2% (3)

5.3.2 Experiment 2: pulses-on versus tickler chains

The comparison of injury incidences between pulses-on and pulses-off might have revealed an effect of electrical pulsing, while keeping all other factors as similar as possible. However, for interpreting the ecological impact of fishing methods, the comparison between pulses-on and tickler chains is also highly relevant. For this comparison, we combined all data samples from pulse gears with the pulses turned on.

Effect of catch method and fish length on spinal injury incidence

The length-frequency distribution for pulses-on is nearly symmetrical, with a maximum at 18 cm. For tickler chains the distribution peaks at 16 cm and is right-skewed (**Figure 5.3A**, **B**). Minor spinal injury incidence was, averaged over all fishing trips, 2.4% and 3% in the pulses-on and tickler-chain samples respectively. Moderate spinal injury

Table 5.4. Generalized additive model selection based on AIC values for the two catch method comparisons (experiment 1 and experiment 2), internal injury types and severities. Selected models used to report potential statistical differences are given in bold. The models that were used to predict the spinal injury probabilities in **Figure 5.2**, **5.3**, and **5.4**, are indicated with an asterisk. The explained deviance is calculated relative to the null model. No moderate spinal injuries were observed in the samples used in experiment 1.

Catch method comparison		Internal injury		Model selection: AIC values					Deviance explained
		Type	Severity	Null	C	s(SL)	C + s(SL)	C + s(SL) + C × s(SL)	
Experiment 1	Pulses-on versus pulses-off	Spinal	Minor	**94.7**	95.6	96.3	97.0*	96.4	0%
			Moderate	NA					
			Severe	**79.3**	81.3	79.9	81.7*	83.5	0%
Experiment 2	Pulses-on versus tickler chains	Spinal	Minor	348.9	349.5	**334.5**	334.5*	335.6	7.7%
			Moderate	56.3	58.1	58.3	60.1	**50.3***	35.7%
			Severe	214.7	201.9	191.1	**183.8***	185.7	23.3%
		Haemorrhage	Minor	387.6	386.2	**363.1**	362.4*	360.8	12.4%
			Severe	193.8	177.7	188.6	**173.5***	175.5	19.9%

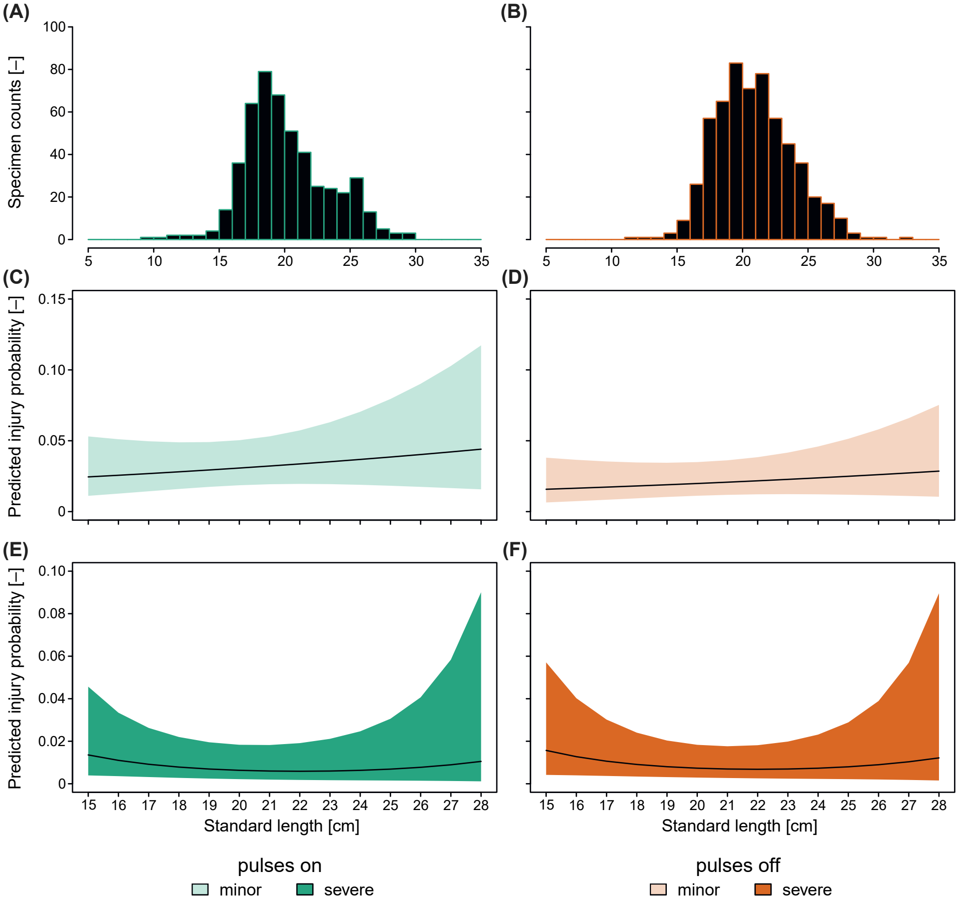

Figure 5.2. Effect of catch method and fish length on spinal injury incidence per severity category for whiting caught with pulse gears with (left panels; *n* = 489) and without (right panels; *n* = 586) electrical stimulus in experiment 1. **(A, B)** Length distributions. Panels **C–F** show the spinal injury probability as function of standard length for **(C, D)** minor and **(E, F)** severe spinal injuries per catch method as predicted with generalized additive models including catch method and standard length (**Table 5.4**). Solid black lines are the fitted values and coloured areas the 95% confidence intervals. No moderate spinal injuries were observed. Neither catch method nor standard length had a significant effect.

incidence was lower but likewise similar between pulse gears and tickler-chain gears: 0.2% and 0.3%, respectively. Severe spinal injury incidence was 0.8% for pulses-on and 2.4% for tickler chains. The incidence of spinal injuries was similar in the cod-end and cover nets (**Supplementary material 5.1**).

The observed overall incidences can, however, not be compared directly, because they could partially result from differences in fish length and other variations related to fishing trip. To assess incidence differences between catch methods, we used statistical

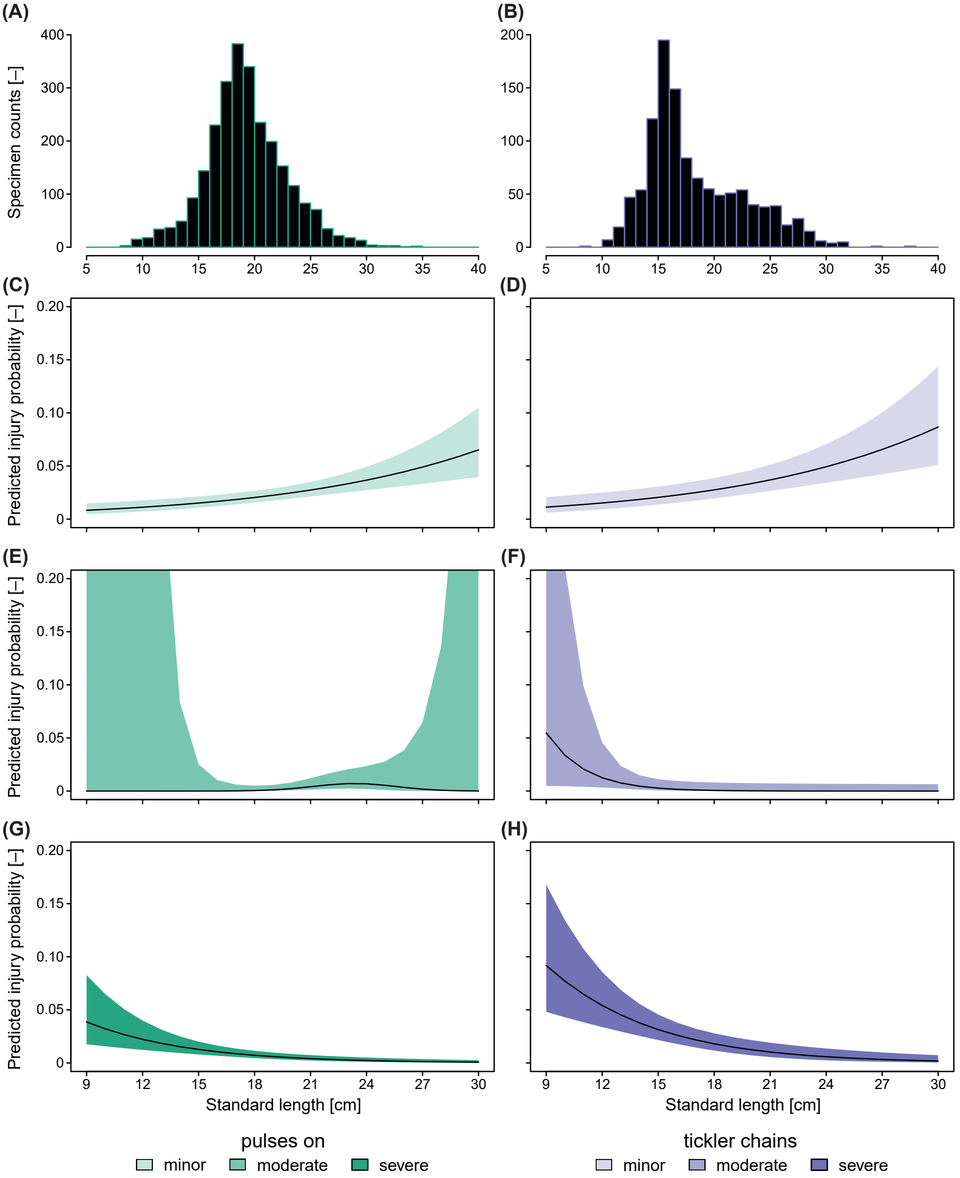

Figure 5.3. Effect of catch method and fish length on spinal injury incidence per severity category for whiting caught with active pulse gears (left panels; *n* = 2616) and with tickler chains (right panels; *n* = 1148) in experiment 2. **(A, B)** Length distributions. **(C–H)** Spinal injury probability as function of standard length for **(C, D)** minor, **(E, F)** moderate, and **(G, H)** severe spinal injuries per catch method as predicted with the selected generalized additive models (**Table 4**). Solid black lines are the fitted values and coloured areas the 95% confidence intervals. Fish-length effects in **(C, D, G, H)** are significant ($p < 0.001$) and severe spinal injury incidence differs significantly between catch methods ($p = 0.002$) **(G, H)**.

models that take fish length and fishing trip into account (**Table 5.4**). For moderate spinal injuries, we found no significant effect of fish length on spinal injury probability (**Figure 5.3E**, **F**). The number of observations was, however, low and variances were high. Minor spinal injury probability increased significantly with fish length for both pulses-on and tickler chains ($p < 0.001$) (**Figure 5.3C**, **D**), whereas severe spinal injury probability decreased significantly with fish length for both catch methods ($p < 0.001$) (**Figure 5.3G**, **H**). For minor and moderate spinal injuries we found no significant effect of catch method (**Figure 5.3C–F**). Severe spinal injury probability was significantly higher for tickler-chain gears than for pulse gears ($z = 3.046$, $p = 0.002$) (**Figure 5.3G**, **H**). In conclusion, fish length had a significant, but opposite effect on injury incidences for minor and severe injuries. Fish-length effects were similar for both catch methods, but the probability of severe injuries was significantly lower for pulse gears than for tickler-chain gears.

Haemorrhage incidence and correlation with spinal injuries

For active pulse gears, 1.8% (40 of 2235) of specimens had a severe haemorrhage and 3.7% (82 of 2235) had a minor haemorrhage (**Table 5.5**). For tickler-chain samples observed incidences were lower: 0.3% for severe and 2.6% for minor haemorrhages (**Table 5.6**). For tickler-chain samples, we found no correlation between haemorrhages and spinal injuries ($p = 0.582$). For pulses-on samples, the presence of haemorrhages showed a significant correlation with spinal injury presence ($p < 0.001$). Animals caught with pulses-on that had a severe haemorrhage, had these in 11 out of 40 cases (27.5%)

Table 5.5. Contingency table showing the frequency of occurrence of haemorrhages and spinal injuries per severity category in the pulses-on samples.

Haemorrhage severity	Spinal injury severity				Total
	None	Minor	Moderate	Severe	
None	2062	39	0	12	2113
Minor	78	2	0	2	82
Severe	23	6	5	6	40
Total	2163	47	5	20	2235

Table 5.6. Contingency table showing the frequency of occurrence of haemorrhages and spinal injuries per severity category in the tickler-chain samples.

Haemorrhage severity	Spinal injury severity				Total
	None	Minor	Moderate	Severe	
None	1054	34	1	26	1115
Minor	28	1	0	1	30
Severe	3	0	0	0	3
Total	1085	35	1	27	1148

in combination with moderate and severe spinal injuries. Likewise, animals that had a moderate or severe spinal injury in the pulses-on samples, had these in 11 out of 25 cases (44%) in combination with a severe haemorrhage. In conclusion, although the incidence of severe haemorrhages was low for both catch methods, in pulses-on samples, but not in tickler-chain samples, the presence of haemorrhages correlated with the presence of spinal injuries, which may indicate a common cause.

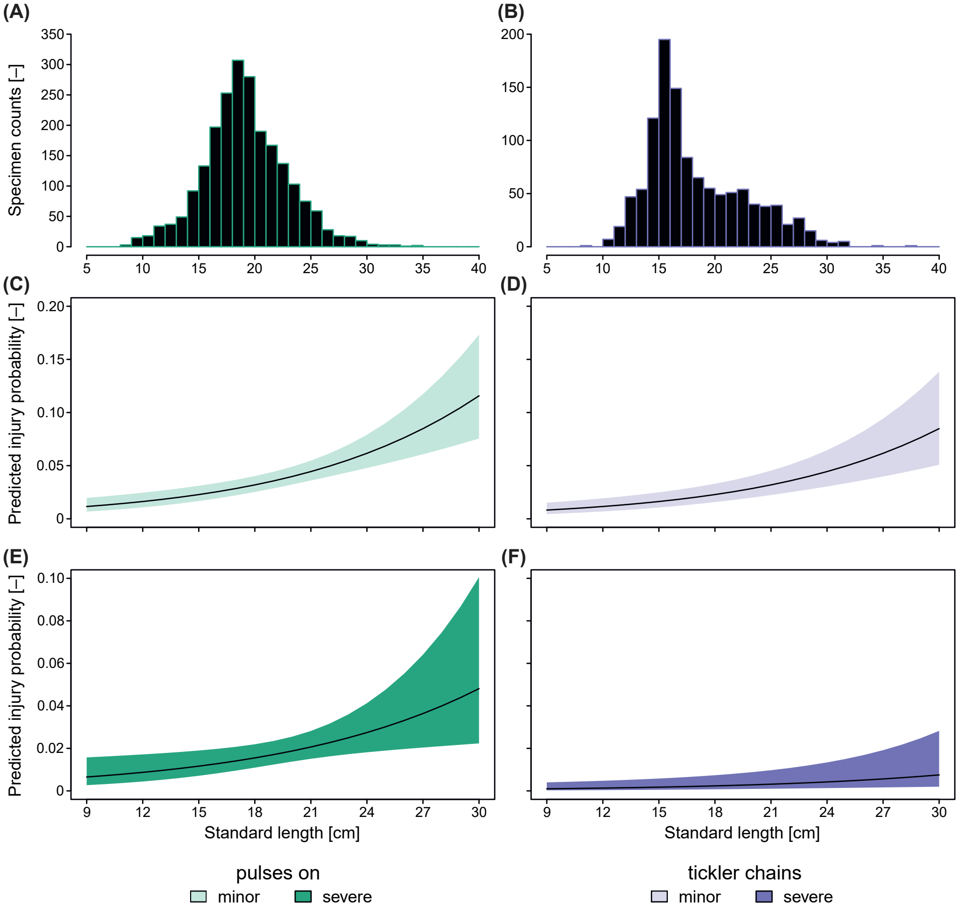

Figure 5.4. Effect of catch method and fish length on haemorrhage incidence per severity category for whiting caught with active pulse gears (left panels; $n = 2235$) and with tickler chains (right panels; $n = 1148$) in experiment 2. **(A, B)** Length distributions. Panels **C–F** show the haemorrhage probability as function of standard length for minor **(C, D)**, and **(E, F)** severe haemorrhages as predicted with the selected generalized additive models (**Table 4**). Solid black lines are the fitted values and coloured areas the 95% confidence intervals. Fish-length effects in **(C, D)** and **(E, F)** are significant ($p < 0.001$ and $p = 0.011$ respectively). Severe haemorrhage incidence differs significantly between catch methods ($p = 0.002$) **(E, F)**.

Effect of catch method and fish length on haemorrhage incidence

To assess differences in haemorrhage incidence between catch methods, we modelled haemorrhage incidences while taking potential effects of fish length and fishing trip into account (**Table 5.4**). For one trip, haemorrhages were not assessed, resulting in a slight smaller sample size and minor differences in length distributions (**Figure 5.4A, B**). Minor and severe haemorrhage probability increased significantly with fish length ($p < 0.001$ and $p = 0.011$, respectively), irrespective of catch method (**Figure 5.4C–F**). The probability of minor haemorrhages did not differ significantly between catch methods, while the probability for severe haemorrhages was significantly lower in the tickler-chain samples than in pulses-on samples ($z = -3.165$, $p = 0.002$). For all, except the largest fish, estimates of severe haemorrhage probability were, however, below 0.02.

Distribution of injuries on the anteroposterior axis

The locations of spinal injuries and haemorrhages on the anteroposterior axis (**Figure 5.5**) do not reveal a clear pattern that might indicate different, distinct causes for injuries between catch methods. For spinal injuries the most salient difference between catch

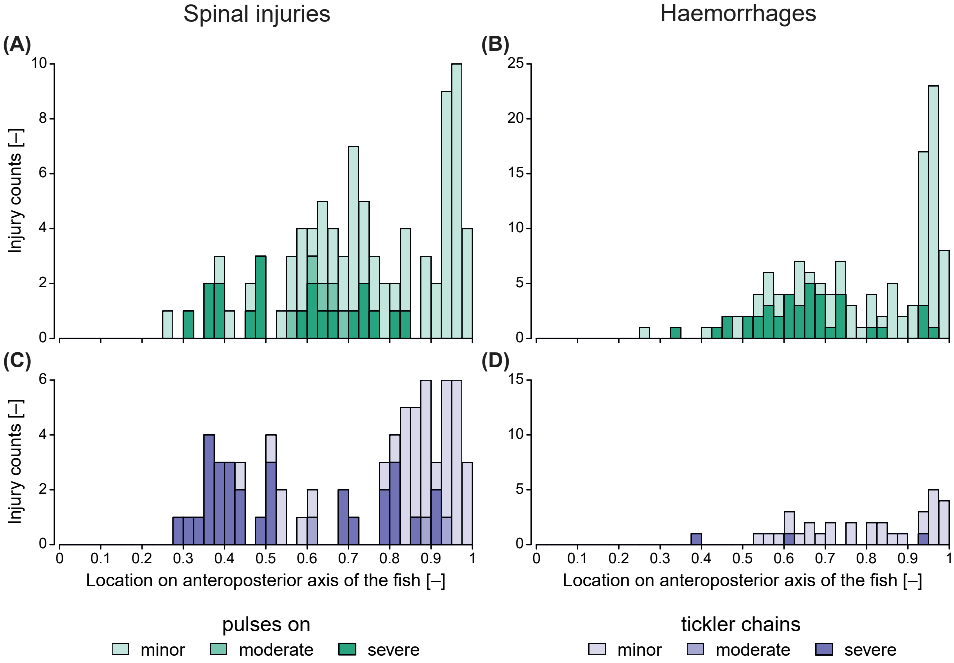

Figure 5.5. Distributions of internal injuries along the anteroposterior axis. **(A, B)** Spinal injuries and haemorrhages for pulses-on samples ($n = 88$ and $n = 125$ respectively). **(C, D)** Spinal injuries and haemorrhages for tickler-chain samples ($n = 70$ and $n = 33$ respectively). Darker colours correspond to increasing injury severity categories. Locations are defined as relative distances from snout (0) to caudal fin (1). The moderate injury category only applies to spinal injuries. Multiple injuries may be present in a single specimen.

methods is a relative lack of minor injuries in the anterior part, up to about 0.75, for tickler-chain gear, whereas severe spinal injuries seem concentrated in the anterior part (0.3–0.4). For pulse gears the locations are more evenly distributed and lack a clear pattern. Injury-location data from the pulses-off treatment in experiment 1 showed a similar pattern, although data were limited due to lower sample size in combination with a low injury incidence (**Supplementary material 5.2**). Minor haemorrhages were distributed evenly in the abdominal, caudal, and ural regions, irrespective of catch method (**Figure 5.5B**, **D**). For tickler chains, the number of severe haemorrhage observations was low. For pulse gears, severe haemorrhages were mostly located in the posterior abdominal and anterior caudal region. As expected from the correlation between incidences of spinal injuries and haemorrhages, the locations of severe spinal injuries and haemorrhages partly coincide for pulse gears.

5.4 Discussion

The main question we addressed is to what extent electrical pulse gears cause internal injuries in whiting. For Atlantic cod, both field data and laboratory experiments showed a high incidence of spinal injuries and related haemorrhages due to electrical pulsing (van Marlen *et al.*, 2014; de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016d, 2016c). Assessing internal injuries in whiting allowed us to determine whether electrically-induced injuries are more general to gadoids, or rather species-specific. Because laboratory experiments with large numbers of whiting are nearly impossible, we performed an extensive analysis of whiting sampled on board commercial vessels.

Our results are straightforward: neither a comparison of samples collected with electrical pulse gears turned on or off, nor a comparison of samples of pulse gears and tickler-chain gears indicated an increased risk of spinal injuries due to electrical pulsing. Most importantly, incidences of spinal injuries were low in samples from pulse gears, and did not match reported high incidences for Atlantic cod (van Marlen *et al.*, 2014; Soetaert *et al.*, 2016d). We observed about 1.0% of fish caught with electrical pulses having moderate or severe spinal injuries. Van Marlen *et al.* (2014) reported a slightly higher incidence of spinal injuries in whiting, but their numbers were based on a small sample size (*n* = 57) from two fishing trips, and were not directly comparable as their fish were larger (27–38 cm) (for photographs, see van Marlen *et al.*, 2011). Moreover, damages were scored without X-radiographs, which is less effective in detecting spinal injuries (e.g., McMichael, 1993). Neither experiment 1, comparing pulse gears with pulses turned or on off, nor experiment 2, comparing pulse gears with tickler-chain gears, revealed pulse-related spinal injuries. Instead, severe spinal injury incidences were significantly lower for the active pulse gear than for the tickler-chain gear.

The reduced incidence of spinal injuries in the active pulse gear coincided with an increased incidence in severe haemorrhages. The presence of haemorrhages was correlated with the presence of spinal injuries for pulses-on samples but not for tickler-chain catches. In the latter case, the absence of a correlation could also be a consequence of the low number of observations, limiting the statistical power. Electrical pulsing thus seems to increase the chance for a haemorrhage, especially in combination with moderate and severe spinal injuries. This could, for example, result from two-sided muscle contractions that dislocate vertebrae and rupture the haemal artery (Hauck, 1949; Snyder, 2003; Soetaert *et al.*, 2019). Similar correlations have been observed in freshwater electrofishing studies (Fredenberg, 1992; Grisak, 1996 as cited in Snyder, 2003; Hollender and Carline, 1994; Holmes *et al.*, 1990; Ruppert and Muth, 1997), depending on the species studied and on electrical stimulus characteristics. In marine electrotrawling, studies on exposed Atlantic cod showed that spinal injuries often, but not always, co-occurred with haemorrhages (de Haan *et al.*, 2016; Soetaert *et al.*, 2016c, 2016b). Absence of a correlation between haemorrhages and spinal injuries has been hypothesised to result from a temporary dislocation of the vertebral column, whereby the intervertebral ligaments return the vertebral column to its normal position after stimulation (Soetaert *et al.*, 2016c). Nevertheless, severe haemorrhage incidence in pulses-on samples was low, in absolute numbers and also in comparison to Atlantic cod.

Low injury incidences relative to Atlantic cod might very well relate to a difference in swimming behaviour when entering a trawl net. Whiting enters higher in trawl nets (Main and Sangster, 1985), which in the case of pulse gear would be at maximally 50 cm, i.e., the distance between the PulseWing and the seabed. As a result, whiting will be exposed to relatively lower electric field strengths. At 25 cm above an electrode, the heterogeneous electric field has a maximal field strength of about 20–30 V m^{-1} (de Haan *et al.*, 2016; ICES, 2020c). In Atlantic cod, field strength thresholds inducing moderate-to-strong muscle contractions have been reported at >37 V m^{-1} and the 50% threshold for inducing spinal injuries was at 80 V m^{-1} (95% CI of 60–110 V m^{-1}) (de Haan *et al.*, 2016). If spinal injuries thresholds would be similar to those in Atlantic cod, most whiting might escape injury-inducing electric field strengths.

The locations of spinal injuries in the fish provided no indication that these were caused by a different mechanism in pulses-on catches than in tickler-chain catches. We did not see the clustering of spinal injury locations, as observed in electrically-exposed Atlantic cod (de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c). However, severe haemorrhages in the pulses-on catches were mostly located in the posterior abdominal and anterior caudal region, which is similar to where electrically-induced injuries in Atlantic cod occur. These findings do not exclude the possibility that electrical pulses play a role in causing severe haemorrhages, but they occur infrequently and coincide with a lower number of spinal injuries. Minor haemorrhages were more abundant in the posterior part of the caudal region and ural region of the fish, irrespective of catch method. Video observations in trawl nets show that this may be due to whiting frequently bumping into the net before ending up in the cod-end (pers. comm. Pieke Molenaar, Wageningen Marine Research). This may also explain the petechiae that were observed, but not quantified, externally on the caudal and ural area of whiting.

In Atlantic cod, direct effects of electrical pulsing could be studied in laboratory experiments (de Haan *et al.*, 2016; Desender *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c). Whiting, however, cannot be acquired from farmed stocks and wild captured specimens have a low survival rate due to e.g., barotrauma (Alexander, 1959; Suuronen, 2005; Povoa *et al.*, 2011; Gilman *et al.*, 2013; Cook *et al.*, 2019). Therefore, we were limited to sampling on board commercial vessels, which may potentially suffer from various biases. To exclude an observer bias, we only using trained researchers following strict sampling protocols. To assess variations between fishing trips, we aimed to collect samples for a large number of fishing trips. This was, however, limited due to the large investments required. Fishing trips lasted five full days and each trip required reservation of space for the observer(s). Although we were able to sample on twelve pulse-gear trips and four tickler-chain trips, one cannot exclude biases due to vessel-specific differences, variations in fishing locations (Hintzen *et al.*, 2021), and/or seasonal variations that might interfere with any potential effect of catch method. Beam trawlers, for example, may use a different number and size of shoe-ticklers and net-ticklers (van Marlen *et al.*, 2014; Depestele *et al.*, 2016, 2019; Rijnsdorp *et al.*, 2021), leading to differences in mechanical impact. Although we only sampled from trawlers with the HFK Engineering PulseWing, which is used by the vast majority of fishers, we have no reason to assume that the result would be different for a DELMECO system (van Marlen *et al.*, 2011, 2014; Soetaert *et al.*, 2015a; de Haan *et al.*, 2016; ICES, 2020c; pers. comm. Harmen Klein Woolthuis of HFK Engineering B.V.). In experiment 1, any potential trip differences were limited as both treatments, sampling with the electrical pulses turned on or off, were done during the same fishing trip at the same location. However, for experiment 2, pulses-on and tickler chains were used by different vessels, as switching gear types at sea or simultaneously fishing with different gears is practically impossible and would also ignore that the gears are typically towed at different speeds (Poos *et al.*, 2020; Rijnsdorp *et al.*, 2021). Our models took both fish-length and trip effects into account to provide means and confidence intervals of incidences across fishing trips, but obviously extrapolation to the fleet should be done with caution.

Both mechanical trauma and electrically-induced trauma may depend on fish length (Dalbey *et al.*, 1996; Ainslie *et al.*, 1998; McMichael *et al.*, 1998; Carline, 2001; Culver and Chick, 2015; de Haan *et al.*, 2016; Veldhuizen *et al.*, 2018). To take length effects into account, we calculated the number of spinal injury observations as proportion injuries per trip per length class and modelled injury incidences with the number of observations as weight factor. This allowed for a direct comparison of incidence levels between pulse gears and tickler-chain gears, irrespective of fish length differences in the samples. The results for spinal injuries were unambiguous: spinal injury incidences for active pulse gears were low and similar to pulse gears without electrical stimulation. Severe spinal injuries incidence was significantly lower for pulse samples than for tickler-chain samples. For severe haemorrhages we observed the opposite pattern. They occurred significantly more often in pulses-on samples than in tickler-chain catches. In all cases, however, incidences were relatively low for both catch methods.

We scored spinal injuries in three, and haemorrhages in two categories. Although we excluded abnormalities that were obviously unrelated to capture, the distinction between natural spinal abnormalities and old and new spinal injuries can be difficult (Snyder, 2003). Especially minor 'injuries' may have resulted from events before capture. Moreover, these minor abnormalities do not necessarily compromise survival, e.g. after escaping from the nets. Therefore, incidences of moderate and severe injuries are most relevant for judging a potential impact of catch method on whiting stocks, and benthic food webs in general. To what extent spinal injuries and haemorrhages result in mortality has not been studied in whiting. Research in a predominantly freshwater electrofishing context (for review, see Snyder, 2003; Soetaert *et al.*, 2015a) indicates that the correlation between electrofishing-injuries and mortality is generally weak (Spencer, 1967; Hudy, 1985; McMichael, 1993; Dalbey *et al.*, 1996; Ainslie *et al.*, 1998; Schill and Elle, 2000; Dolan *et al.*, 2002; Dolan and Miranda, 2004; Miranda and Kidwell, 2010) and injuries may heal over time (Spencer, 1967; Fredenberg, 1992; Dalbey *et al.*, 1996; Schill and Elle, 2000; Holliman *et al.*, 2010). Still, fish may have reduced food-intake (de Haan *et al.*, 2016) and growth rates (Dalbey *et al.*, 1996; Ainslie *et al.*, 1998), and may display abnormal swimming behaviour (Spencer, 1967; Hudy, 1985; de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c), which could increase their susceptibility to predation. In the present study, severe spinal injuries were observed infrequently and significantly less for pulse gears than for tickler-chain gears. Severe haemorrhages were also infrequently observed, but more often in pulses-on catches, especially for larger whiting. For smaller specimens the differences were smaller.

To assess ecological effects of fishing methods, the fate of small fishes (target and non-target) is of special interest. Large specimens are retained in the nets anyhow and are landed for commerce. Small specimens could survive either by escaping from the nets or after selection on board and returned as discards (Sangster *et al.*, 1996; Suuronen, 2005). However, in the latter case survival probability is low (Depestele *et al.*, 2014) and nowadays specimens smaller than the minimum conservation reference size need to be landed anyways as part of the Landing Obligation (European Parliament and Council, 2013; European Commission, 2018, 2019). Severe injuries in small specimens that escape through the meshes could compromise survival and are, therefore, especially important to assess the broader impact on fish communities. To increase the number of small fish in our samples and specifically include fish that would normally escape, we used cover nets on several trips. We found no clear differences between fish from cover nets or the cod-end (**Supplementary material 5.1** and **5.3**). In experiment 2, we found similar length effects for the two catch methods. Minor spinal injuries increased with fish size, which might be due to accumulation of natural, non-lethal abnormalities or healed injuries over a lifetime. Severe injuries, which are more likely related to immediate capture effects, affect smaller fish to a larger extent. This is different from Atlantic cod exposed in the laboratory, where specimens of an intermediate size had an elevated spinal injury incidence (de Haan *et al.*, 2016). In experiment 1, no length effects were observed, presumably due to the smaller sample sizes. It is not directly obvious how the observed length effects relate to electrical and/or mechanical stresses. The mechanical load imposed by the tickler-chain gear is expected to be larger than by pulse gears. Tickler-chain gears consist of multiple chains attached to the shoes and net, and a ground rope which are dragged perpendicularly to the towing direction. In contrast, pulse gears generate electric fields between electrode arrays that are dragged in parallel to the towing direction (Rijnsdorp *et al.*, 2020). Furthermore, tickler-chain gears are towed at higher speeds than pulse gears (Poos *et al.*, 2020), thus tickler-chain gears cover a larger area and have larger catch volumes that also contain more debris and hard-bodied invertebrates (van Marlen *et al.*, 2014). This may explain a higher incidence of spinal injuries in tickler-chain gears but does not explain the observed length effects, or the contrasting finding for spinal injuries and haemorrhages. Most importantly, we found no evidence that pulse gears would affect smaller fish to a larger extent than tickler-chain gears, which might indicate a larger impact on whiting stocks in the North Sea.

In conclusion, our analyses of injury incidences in whiting dismiss electrically-induced spinal injuries as a general problem for gadoids in electrical pulse fishing. Both in tickler-chain samples and pulse-gear samples injury incidences were low. Moreover, pulse gears with pulses turned or of off showed similar injury patterns. Although electrical pulsing may slightly increase the risk for severe haemorrhages, it reduces the risk for severe spinal injuries. This implies that for whiting, advantages of pulse gears, for example lower habitat disturbance, lower fuel consumption, and increased selectivity may tip the balance in favour of electrical pulse fishing.

5.5 Statements

5.5.1 Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5.5.2 Author contributions

All authors contributed to the conception and design of the study; PGB, WSMV and RPMP processed the dead fish; MJL programmed the database program; PGB and WSMV collected the data from the X-ray and dissection images. PGB, ADR, and WSMV performed the statistical analyses. All authors interpreted the data and discussed the results; PGB drafted the initial manuscript and figures, with contributions by ADR, JLvL, WSMV, RPMP, and MJL. All authors contributed to the critical revision of the manuscript and figures, and approved the final version.

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Supplementary material

Supplementary material 5.1. Spinal injury incidences of whiting in cod-end and cover-net catches.
Supplementary material 5.2. Location of internal injuries in whiting caught by pulse gears without electrical stimulation.
Supplementary material 5.3. Length-frequency distributions of whiting samples from the cod-end and cover net.

Supplementary Material 5.1. Spinal injury incidences of whiting in cod-end and cover-net catches.

Table S5.1A. Spinal injury incidences in whiting when, besides the cod-end, also cover nets were used. For V1, minor injuries incidence are combined for cod-end and cover-net samples. Injury incidences within these net parts are shown in **Table S5.1B**.

Vessel	Year	Week	Treatment	Number of animals		Injury incidence per severity (*n*)		
				Cod-end	Cover	Minor	Moderate	Severe
V1	2016	29	Pulses on	48	333	3.6% (14)	0%	0%
			Pulses off	67	198	3.8% (10)	0%	0%
V6	2017	24	Pulses on	0	609	2.3% (14)	0.2% (1)	0%
		33		0	275	4% (11)	0%	0.7% (2)

Table S5.1B. Minor spinal injury incidences in whiting per net part origin, i.e. cod-end and cover net. No moderate and severe spinal injuries were observed.

Vessel	Year	Week	Treatment	Number of animals		Minor injury incidence per net part (*n*)	
				Cod-end	Cover	Cod-end	Cover
V1	2016	29	Pulses on	48	333	4.2% (2)	3.6% (12)
			Pulses off	67	198	6% (4)	3% (6)

Supplementary material 5.2. Location of internal injuries in whiting caught by pulse gears without electrical stimulation.

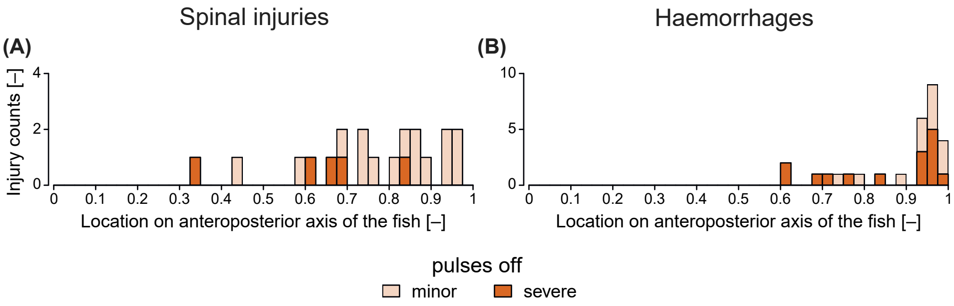


Figure S5.2. Distributions of internal injuries along the anteroposterior axis. **(A)** Spinal injuries and **(B)** haemorrhages for pulses-off samples (*n* = 20 and *n* = 28 respectively). Darker colours correspond to increasing injury severity categories. Locations are defined as relative distances from snout (0) to caudal fin (1). Moderate spinal injuries were not observed. Multiple injuries may be present in a single specimen.

Supplementary material 5.3. Length-frequency distributions of whiting samples from the cod-end and cover net

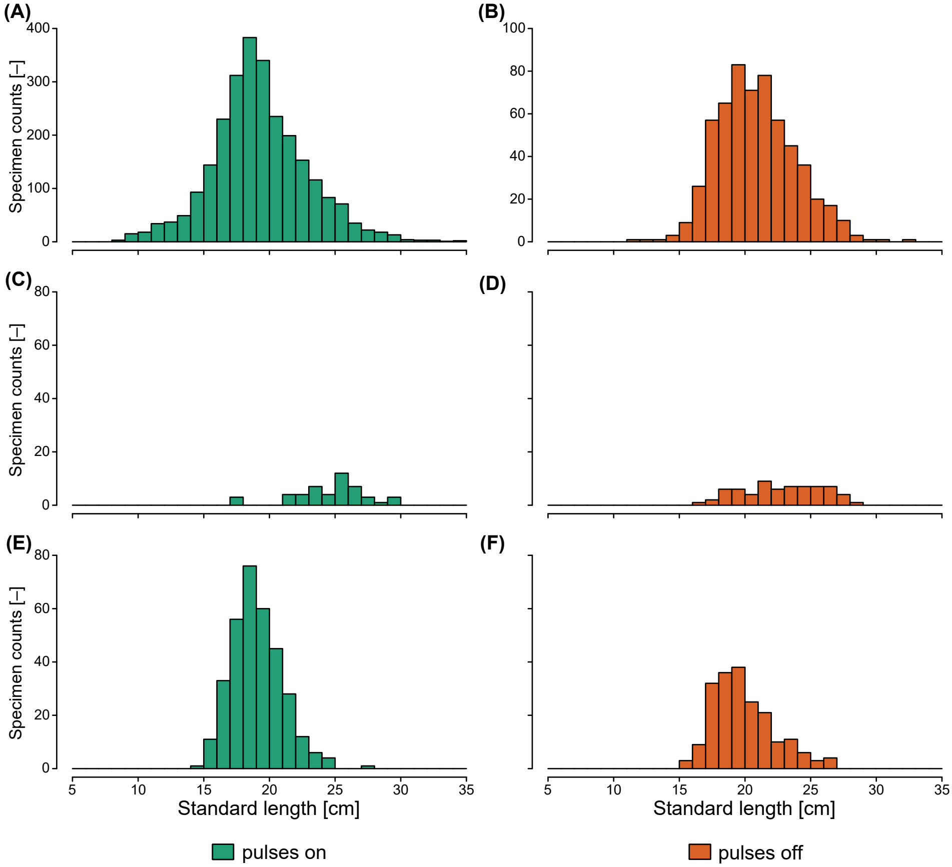


Figure S5.3A. Length-frequency distributions of **(A,B)** all pulses-on (*n* = 2616) and pulses-off (*n* = 586) samples respectively, **(C,D)** all pulses-on (*n* = 48) and pulses-off (*n* = 67) samples from the 80 mm diamond-shaped cod-ends of V1 respectively, and **(E,F)** all pulses-on (*n* = 333) and pulses-off (*n* = 198) samples from the 40 mm diamond-shaped cover nets of V1 respectively.

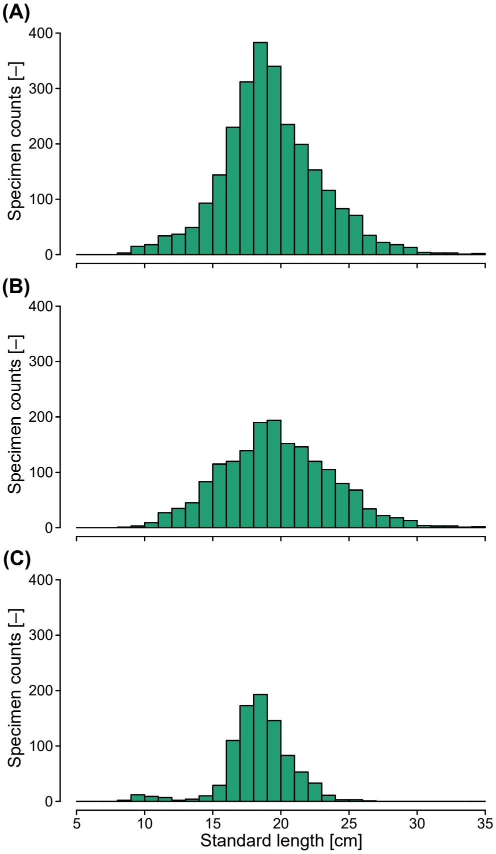
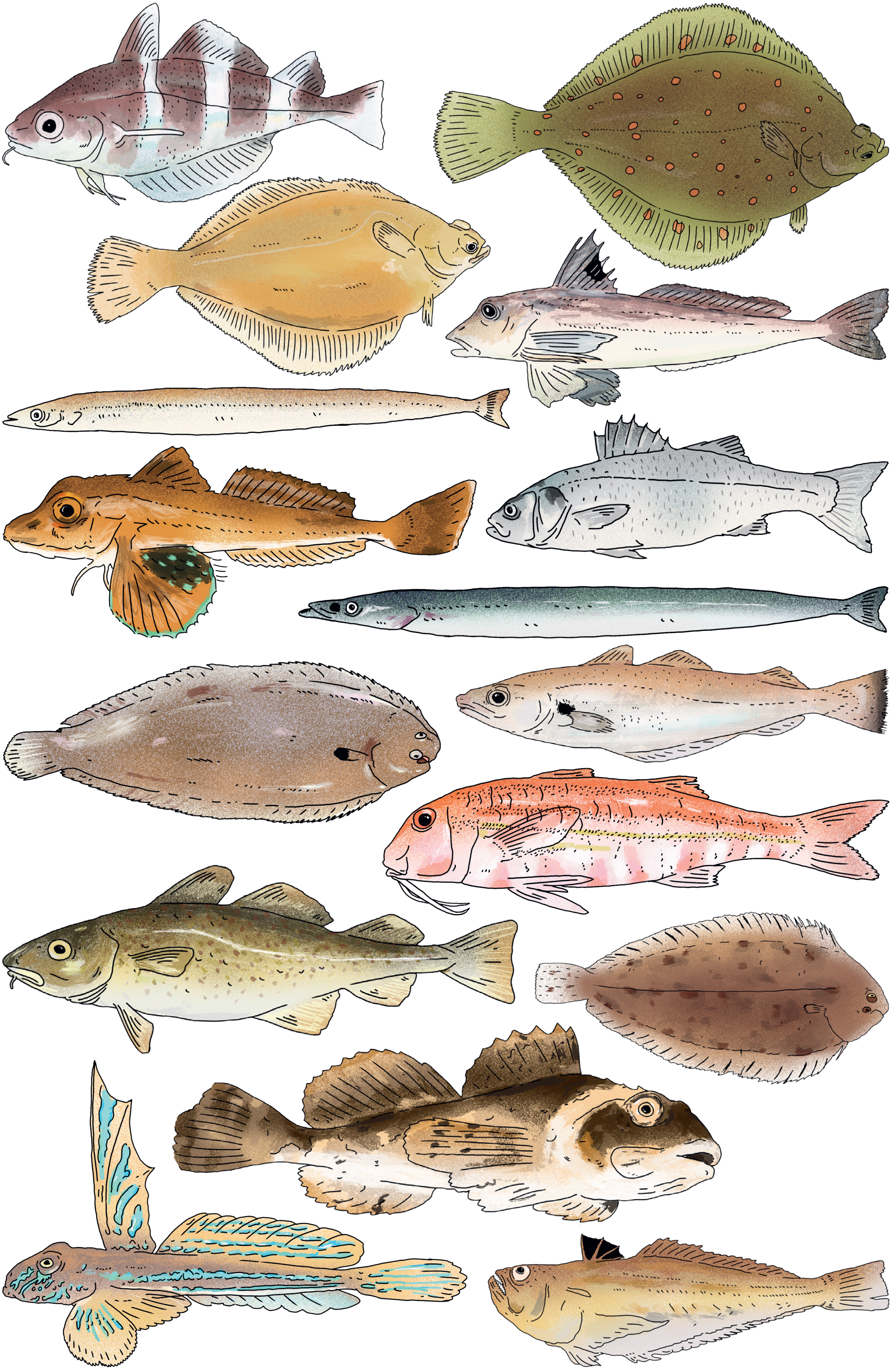


Figure S5.3B. Length-frequency distributions of **(A)** all pulses-on samples ($n = 2616$), **(B)** all pulses-on samples minus the V6 samples ($n = 1732$), and **(C)** the pulses-on samples from the two V6 trips combined ($n = 884$) , which were collected from the 40 mm diamond-shaped cover nets that spaciously fitted over the 80 mm cod-ends.



Chapter 6

Spinal injuries in marine fish species caught by bottom trawls with tickler-chain and electrical-pulse gears

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Abstract

Bottom trawling for common sole (*Solea solea*) in the North Sea conventionally utilises tickler chains in front of the nets to drive fish from the seafloor. An alternative is the use of pulsed electric fields, which may reduce the ecological footprint, but comes with a concern about a potential high risk of spinal injuries, as shown in Atlantic cod (*Gadus morhua*). To quantify this risk, we studied spinal injuries in sixteen, widely different, fish species from catches of tickler-chain trawlers and electrical-pulse trawlers. Sampled species included common sole, dab (*Limanda limanda*), European plaice (*Pleuronectes platessa*), solenette (*Buglossidium luteum*), Atlantic cod, bib (*Trisopterus luscus*), whiting (*Merlangius merlangus*), grey gurnard (*Eutrigla gurnardus*), tub gurnard (*Chelidonichthys lucerna*), lesser sandeel (*Ammodytes tobianus*), greater sandeel (*Hyperoplus lanceolatus*), bullrout (*Myoxocephalus scorpius*), dragonet (*Callionymus lyra*), European seabass (*Dicentrarchus labrax*), lesser weever (*Echiichthys vipera*), and striped red mullet (*Mullus surmuletus*). Furthermore, to distinguish mechanically and electrically-induced injuries, we also compared, for a subset of species, injuries in samples from pulse gears with electrical pulses either turned on or off. Severity of spinal injuries and their location along the anteroposterior fish axis were quantified from X-radiographs. Except for Atlantic cod and sandeels, spinal injury probability was low (<2.5%), irrespective of severity level and catch method. In sandeels, major spinal injuries occurred in, on average, 13% of pulses-on samples and in 33% of tickler-chain samples, suggesting no evidence for electrically-induced injuries. In Atlantic cod, 40% had major spinal injuries in pulses-on samples versus 1% in tickler-chain samples. Both the location of injuries in the pulses-on samples and fish-length dependency of injury incidences, match findings for Atlantic cod in laboratory experiments. Overall, our results show that electrically-induced spinal injuries as present in Atlantic cod are not found in a wide range of other bycatch species of common-sole-targeting bottom trawling. Apart from Atlantic cod, pulse trawling is therefore unlikely to impose increased mortality on studied fish populations compared to the tickler-chain technique.

Keywords: beam trawling; bottom trawling; bycatch species; electrotrawling; electrical pulse fishing; *Solea solea*; spinal injury; North Sea

6.1 Introduction

Wild capture fishery methods may induce injuries in fishes (Veldhuizen *et al.*, 2018; Cook *et al.*, 2019; Tveit *et al.*, 2019; Brinkhof *et al.*, 2021), for example spinal injuries due to the mechanical impact of fishing gears. For large fish, such injuries are ecologically irrelevant as they are retained on board anyway, but injured smaller specimens may be hampered in their escape capabilities through the trawl meshes, and injuries may increase predation risk in escapees leading to increased fishing mortality (Chopin and Arimoto, 1995; Suuronen, 2005; Broadhurst *et al.*, 2006; Suuronen and Erickson, 2010; Gilman *et al.*, 2013; Raby *et al.*, 2014; Wilson *et al.*, 2014). This may subsequently lead to disturbed food web structures affecting population dynamics of other species (Kaiser *et al.*, 2002; Hiddink *et al.*, 2011; van Denderen *et al.*, 2013; Collie *et al.*, 2017). Quantification of internal injuries in fishes caused by different catch methods is therefore essential to assess the potential negative impacts of fisheries on population dynamics and the ecosystem.

Towed demersal fishing gears are used globally to capture demersal and benthic organisms (Amoroso *et al.*, 2018; Cashion *et al.*, 2018; Watson and Tidd, 2018; Zeller *et al.*, 2018). In the North Sea, bottom trawls with 80 mm meshes are used to target the flatfish species common sole. Conventionally, tickler-chains are dragged in front of the trawl, perpendicularly to the towing direction, to drive the fish from the seafloor into the net (Rijnsdorp *et al.*, 2008). This method is, however, criticised for its negative environmental effects (de Groot, 1984; van Beek *et al.*, 1990; Lindeboom and de Groot, 1998; Paschen *et al.*, 2000; Poos *et al.*, 2013; Uhlmann *et al.*, 2014). A promising alternative is the use of electrical pulses, generated by electrode arrays that are dragged in parallel to the towing direction (Soetaert *et al.*, 2015a). The electric field induces involuntary muscle contractions that immobilise fishes in front of the trawl, followed by capture over the ground rope, into the nets (de Groot and Boonstra, 1970; Stewart, 1977; Soetaert *et al.*, 2019). This so-called pulse trawling has several advantages over tickler-chain trawling, including increased selectivity (van Marlen *et al.*, 2014; Poos *et al.*, 2020), higher discard survival rates (van der Reijden *et al.*, 2017), reduced fuel consumption (van Marlen *et al.*, 2014; Poos *et al.*, 2020), lower discard rates (van Marlen *et al.*, 2014), reduced physical disturbance of the benthic ecosystem (Depestele *et al.*, 2016, 2019; Rijnsdorp *et al.*, 2020, 2021a), reduced impact on benthic organisms (Soetaert *et al.*, 2015b, 2016a; Bergman and Meesters, 2020; Boute *et al.*, 2021) and higher revenues (Batsleer *et al.*, 2016). Replacing mechanical stimulation in beam trawls with electrical stimulation in pulse trawls could thus substantially reduce the ecological footprint of bottom trawling for common sole (Rijnsdorp *et al.*, 2020). A concern, however, is the presence of pulse-induced spinal injuries in Atlantic cod and the possibility of such injuries in other fish species that come into contact with pulse-trawl gears (e.g. de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016d; Quirijns *et al.*, 2018).

Both tickler-chain gears and pulse-trawl gears may inflict spinal injuries. In beam trawling with tickle-chains, spinal injuries can be caused by the mechanical load caused via gear components and handling on deck, which can be affected by towing speed, catch composition and catch volume (ICES, 2018). In pulse trawling, in addition to mechanically-induced trauma, the electrical stimulus may induce excessive muscle contractions in fish that are close to the electrodes, leading to spinal injuries. So far, only a few fish species have been studied for muscle-cramp-induced injuries in the context of common-sole-targeting pulse trawling. Spinal injuries have not been found in common sole and European seabass (*Dicentrarchus labrax*) exposed in controlled laboratory conditions (Soetaert *et al.*, 2016b, 2018). However, in the laboratory, pulse-induced spinal injuries occurred in 0–37% of exposed Atlantic cod (de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c), depending on fish size and electric field strength. In Atlantic cod caught with pulse gears or electrified benthos release panels, relatively high injury incidences (7–11%) have been reported (van Marlen *et al.*, 2014; Soetaert *et al.*, 2016d). Van Marlen *et al.* (2014) reported a single injured whiting (*Merlangius merlangus*) out of 57 filleted specimens sampled from pulse gear catches, but we found, in **Chapter 5**, that spinal injuries in whiting were rare and primarily due to mechanical impact. For many other fish species that may come into contact with bottom trawl gears in the North Sea (ICES, 2020a) the incidence of spinal injuries is unknown. To address concerns regarding spinal injuries in different species, we extensively sampled catches on board commercial pulse and tickler-chain trawlers targeting common sole in the North Sea.

Fish were sampled on pulse trawlers, with electrical stimulation either turned on or off, and on conventional beam trawlers using tickler chains. By comparing the spinal injury incidences between catch methods, we pinpoint whether species are susceptible to either mechanically or electrically-induced injuries. After landing, we X-radiographed all fish to quantify spinal injuries in discrete severity classes and to measure locations of injuries along the anteroposterior axis. Such distributions in injury locations may reveal a specific cause of injuries, as has been previously suggested for Atlantic cod, where electrical-pulse-induced spinal injuries are typically located in a narrow range in the anterior part of the caudal region (de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c). In addition, we analysed how injury incidences vary with fish size, which is relevant for the discussion of ecological implications of different catch methods, as small fish have a chance of escaping the nets before hauling.

6.2 Materials and methods

6.2.1 Collection of animals

Fishes were sampled from catches on board commercial vessels targeting common sole by scientists from Wageningen Marine Research and the Experimental Zoology Group (EZO) of Wageningen University & Research (**Table 6.1**) as described in **Chapter 5**. For two fishing trips fish were sampled directly after landing, as too many discards were retained in the presence of an independent observer. Fishing trips lasted five days, from Sunday evening to Friday morning. Except for the 3–9 hours required to reach the fishing grounds, fishing continued day-and-night in hauls lasting about two hours. After a haul, fish were unloaded in hoppers on deck and processed while the next haul started immediately. Sampling occurred during as many hauls as possible before handling by fishermen. Fish samples were stored in sealed plastic bags on ice. After landing, all fish were stored at –20 °C in freezer facilities at Wageningen University.

Specimens were collected during sixteen fishing trips made by nine electrical pulse trawlers, and during five trips by three tickler-chain trawlers (**Table 6.1**). Fishes were randomly sampled, except for Atlantic cod, for which all specimens were collected to prevent a potential sampling bias, as pulse-induced injuries were visible due to dark skin discolouration (de Haan *et al*., 2016; Soetaert *et al*., 2016c, 2016b). Two vessels (V2 and V9) fished for one tow with the pulses turned off on both starboard and portside. A third vessel (V1) fished with the pulses turned off, either on starboard or portside, for seven tows. For the comparison of pulses-on versus pulses-off, we aimed to obtain similar numbers of specimens.

Pulse trawlers used a PulseWing from HKF Engineering B.V. with a Pulsed Bipolar Current waveform (Soetaert *et al*., 2019) and tickler-chain beam trawlers used a SumWing (Rijnsdorp *et al*., 2021a). All vessels used the towing speed that they would use without sampling, which was typically lower for pulse gears than for tickler-chain gears (van Marlen *et al*., 2014; Depestele *et al*., 2019; Poos *et al*., 2020; Rijnsdorp *et al*., 2020). Electrical-pulse settings were highly similar between trips and vessels and matched those of 33 pulse trawlers in the fleet using the HFK system (ICES, 2020b). The vessels V10, V11, and V12 used conventional tickler-chain beam trawls with 6–8 shoe tickler chains and 12–14 net tickler chains respectively.

All vessels used 80 mm diamond-shaped mesh cod-ends, which are the standard in common-sole-targeting trawl fisheries (Rijnsdorp *et al*., 2008). To increase sample size with small fish that might have escaped from the cod-end, pulse trawler V1 was equipped with 40 mm mesh cover nets that spaciously fitted over the cod-end and specimens

Table 6.1. Trawler sampling overview per vessel (anonymised coding) and trip with catch year and week, catch method, number of collected species and animals, towing speed, and electrical-pulse settings. Peak amplitude is at the seafloor. Details on number of specimens per species and trip as well as cod-end and cover-net samples are provided in **Supplementary material 6.1**.

Vessel	Year	Week	Catch method	Number of species	Number of animals	Towing speed (mean ± SD) [kn]	Electrical pulse settings (mean ± SD)			
							Peak amplitude [V]	Frequency [Hz]	Width [µs]	Duty cycle [–]
V1	2016	29	Pulses on	12	1047	5.1 ± 0.1	54.3 ± 2.9	30 ± 0	349.3 ± 2.5	2.1%
			Pulses off	5	592	5.1 ± 0.1	NA			
V2		41	Pulses on	2	566	5.0 ± 0.1	55.5 ± 3.0	30 ± 0	350 ± 0	2.1%
	2017	36		1	11	5.0 ± 0.1	58.3 ± 3.1	30 ± 0	340 ± 0	2.0%
	2018	4		6	108	5.0 ± 0.1	54.3 ± 3.1	30 ± 0	300 ± 0	1.8%
			Pulses off	6	1079	5.0 ± 0.1	NA			
		36	Pulses on	2	107	5.0 ± 0.1	58.1 ± 3.0	30 ± 0	330 ± 0	2.0%
		46		1	64	5.0 ± 0.1	55.6 ± 3.0	30 ± 0	350 ± 0	2.1%
V3	2017	6		2	126	4.7 ± 0.1	58.7 ± 3.0	30 ± 0	300 ± 0	1.8%
V4		7		4	239	4.9 ± 0.1	55.3 ± 2.9	30 ± 0	350 ± 0	2.1%
V5		24		13	2916	4.9 ± 0.3	58.3 ± 2.9	30 ± 0	350 ± 0	2.1%
		33		12	2001	4.9 ± 0.3	58.3 ± 3.1	30 ± 0	350 ± 0	2.1%
V6		24		7	573	5.0 ± 0.1	56.0 ± 3.1	30 ± 0	335.3 ± 5.0	2.0%
		44		6	404	5.0 ± 0.1	55.5 ± 3.0	30 ± 0	330 ± 0	2.0%
V7	2018	4		5	238	5.0 ± 0.1	54.6 ± 3.1	30 ± 0	350 ± 0	2.1%
	2019	5		2	112	5.0 ± 0.1	54.8 ± 3.1	30 ± 0	350 ± 0	2.1%
V8	2018	6		5	178	5.0 ± 0.1	54.8 ± 3.2	30 ± 0	330 ± 0	2.0%
V9		8		7	286	5.0 ± 0.1	56.1 ± 2.9	22.5 ± 0	390 ± 0	1.8%
			Pulses off	3	1318	5.0 ± 0.1	NA			
V10		23	Tickler chains	11	1432	6.1 ± 0.1				
		26		12	2084	6.1 ± 0.1				
V11		47		9	1276	6.5 ± 0.3				
	2019	9		3	302	6.5 ± 0.3				
V12		8		1	26	6.4 ± 0.5				

were sampled from both the cod-ends and cover nets, for the pulses-on and pulses-off catch method. In addition, pulse trawler V5 fished with trouser nets equipped with 40 mm mesh covers (from where specimens were sampled).

Collection of fish was approved by the Animal Welfare Body of Wageningen University, the Animal Ethics Committee of Wageningen University & Research, and the Central Authority for Scientific Procedures on Animals (application number AVD1040020184945). Atlantic cod, common sole, European plaice, European seabass, and whiting have a legal European Union (EU) regulated minimum landing size of 35, 24, 27, 42, and 27 cm (total length) in the North Sea respectively (Council of the European Union, 1998; European Parliament and Council, 2019). In case collecting undersized specimens could not occur under the Landing Obligation as part of the EU Common Fisheries Policy due to phased implementation (European Parliament and Council, 2013; European Commission, 2018, 2019), collection occurred with permission of the authorities via derogations granted by the Netherlands Enterprise Agency. The use of cover nets for experimental purposes was approved by the Netherlands Enterprise Agency.

6.2.2 X-ray analysis

For X-ray photography we followed the methodology described in **Chapter 5**. Briefly, we defrosted and subsequently X-rayed the fish using a Philips X-ray machine (SRM 0310 tube s/n 923436) with a 46401G housing (2.3 mm Al total filtration), Philips XD6028 collimator (0.2 mm Al total filtration), generator Philips Super CP 80 (s/n 953031), Philips Super CP 50 control panel, and a Philips Optimus M200 frame. Flatfish species were X-rayed laterally only, other species were also X-radiographed dorsoventrally, except for fish collected from V1. Depending on fish size, X-ray settings varied in the range of 40–71 kV and 32–71 mAs, and images were captured by either a 35.2 x 42.8 cm phosphor plate (4020 x 4892 px, pixel size 87.5 μm, 12 bit) or a 23.8 x 29.7 cm mammography phosphor plate (5440 x 6776 px, 43.75 μm, 12 bit) in combination with a Konica Minolta Regius 110HQ digitizer. Distance between X-ray source and plate was 127 cm. After X-radiography, fish standard length was measured to the nearest millimetre (Rabone Chesterman No 47R mounted on a measuring board).

6.2.3 Spinal injury category and scoring system

For scoring spinal injuries, we followed the methodology described **Chapter 5**. In short, spinal injuries were categorised based on (i) own observations during an exploratory assessment, (ii) injuries scored in freshwater electrofishing studies (Fredenberg, 1992;

Hollender and Carline, 1994; Dalbey *et al.*, 1996; Snyder, 2003), and (iii) those reported in laboratory exposure experiments in the context of marine electrotrawling (de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c). Spinal injuries were scored in three categories: minor, moderate, and severe. Examples of these injury categories for a common sole and an Atlantic cod are provided in **Figure 6.1**. Minor spinal injuries were deformations of one or multiple vertebrae including a minor subluxation. Moderate spinal injuries were a subluxation or compression of several vertebrae (i.e. spinal misalignment) with

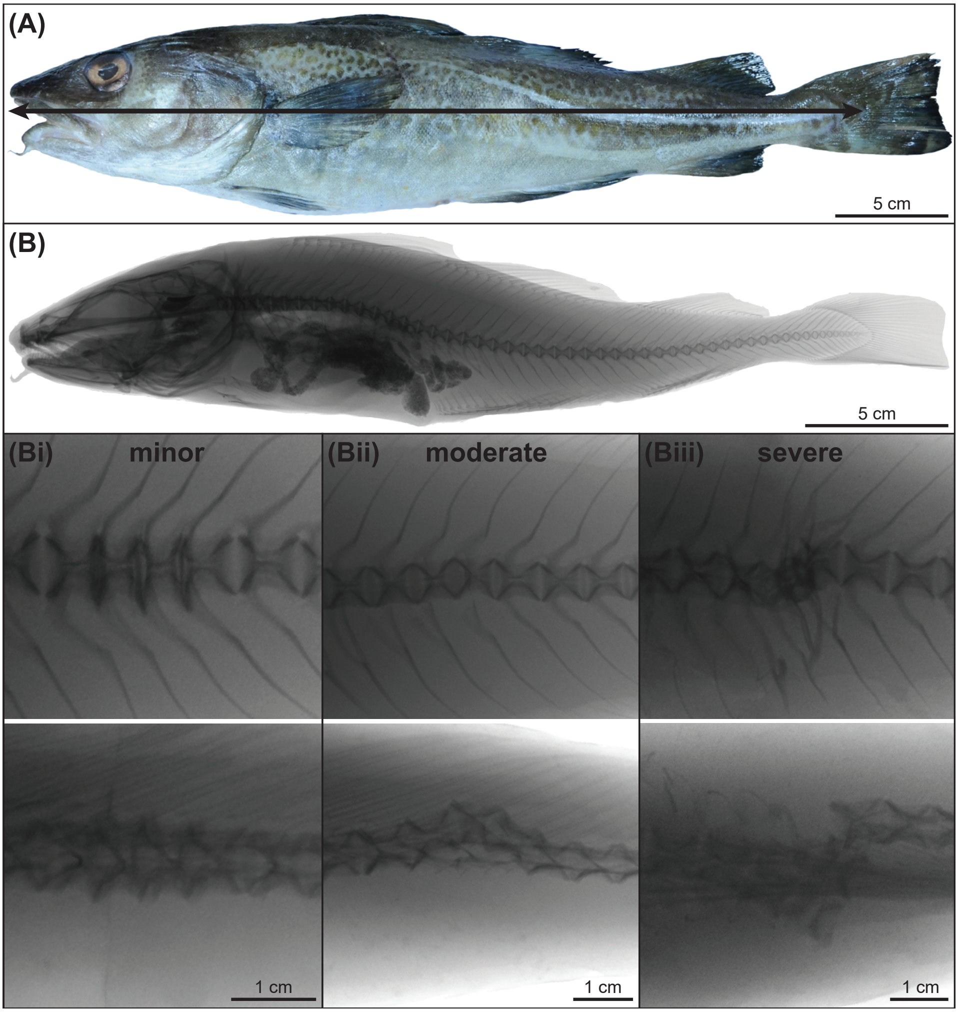

Figure 6.1. Spinal injury categorisation types in Atlantic cod. **(A, B)** and common sole **(C, D)**. **(A, C)** The location of spinal injuries was quantified on the anteroposterior axis of the fish relative to the snout and caudal fin as indicated by the black double arrow. **(B, D)** Lateral X-radiograph of a specimen without injury. Spinal injuries were subdivided into **(Bi, Di)** minor, **(Bii, Dii)** moderate, and **(Biii, Diii)** severe (top and bottom images are lateral and dorsoventral X-radiographs of the same fish respectively; for flatfish only lateral X-radiographs were made).

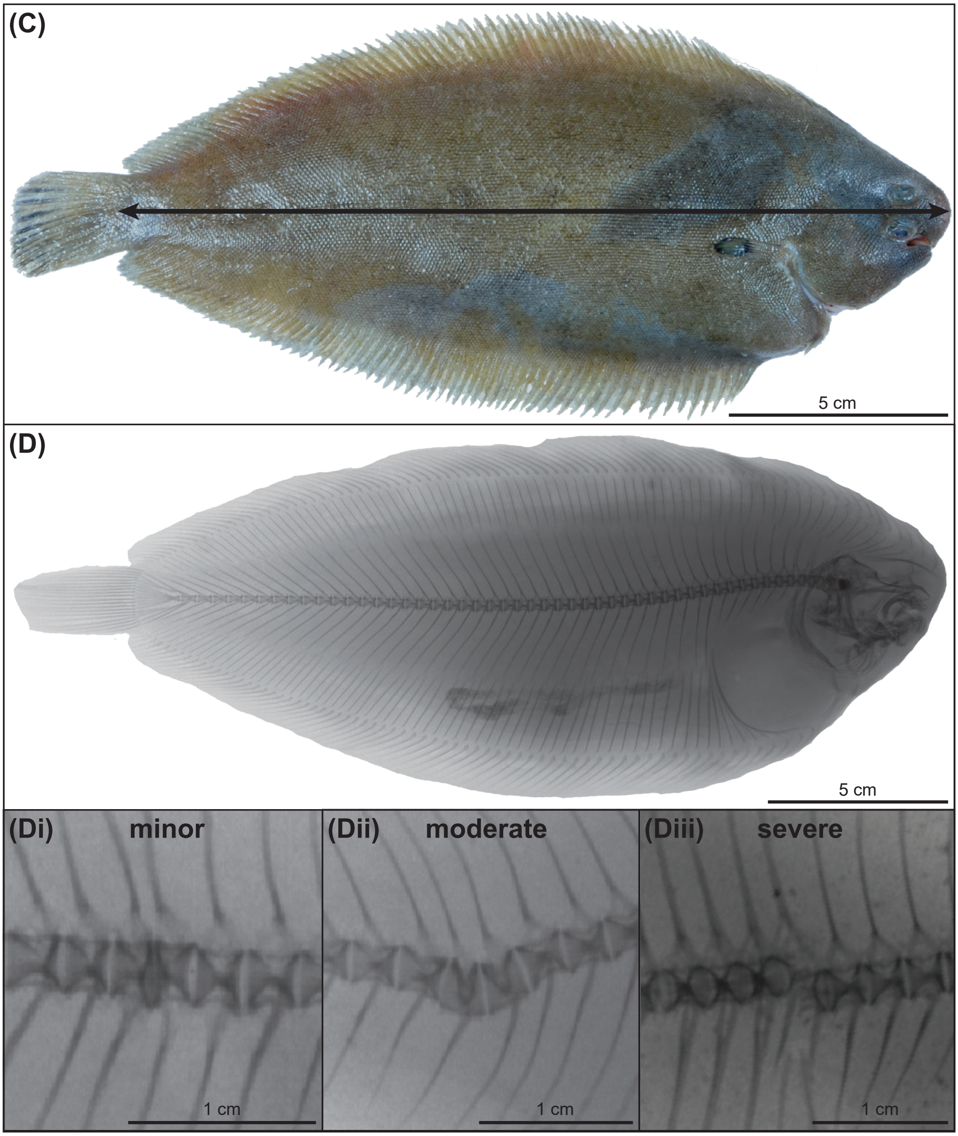

Figure 6.1 (continuation).

minor fractures only. Severe spinal injuries were fractured and/or clearly dislocated vertebrae. Skeletal deformities that were clearly unrelated to acute injuries, such as the presence of additional spines, spinal curvature linked to developmental luxation, and block vertebrae, were not taken into account (**Chapter 5**). To score the presence and location of spinal injuries on the anteroposterior axis of the fish, we used a custom-made software database system in `Python` (Python Software Foundation, n.d.), in combination with `OpenCV`, and `SQLiteManager` in Mozilla Firefox. The database system coupled all X-radiographs to the relevant metadata, and recorded user input indicating the tip of the snout, base of the tail (posterior end of the mid-lateral portion of the hypural plate) as well as locations and severities of spinal injuries.

6.2.4 Data analyses

Raw data consist of observed injury incidences for all species per catch method and injury severity level. To compare spinal injury incidences between pulses-on and tickler-chain catches, we used the injury probability by trip as response variable. Although mixed-effect models including random effects for the trip are the preferred approach, we chose to use a simplified model because of the rather unbalanced distributions of fish sizes across trips. We modelled observed incidences using generalized additive models (Wood, 2017), which allow for non-linear dependencies of incidences on fish length (i.e. smooths), as found in laboratory-exposed Atlantic cod (de Haan *et al.*, 2016). We first assessed spinal injury probability in a null model (i.e., intercept only) to estimate the mean with confidence interval using a logit link for the binomially-distributed response:

$$\text{(i)} \qquad \mathrm{P}_j \sim Bin(1, p_j), logit(p_j) = \alpha,$$

where P_j corresponds the injury probability observed in trip (*j*), and *α* is the overall intercept. To test the effect of catch method, we included the covariable *C* (pulses-on, pulses-off; pulses-on, tickler chains) in model (ii):

$$\text{(ii)} \qquad \mathrm{P}_j \sim Bin(1, p_j), logit(p_j) = \alpha + \beta_1 C_j.$$

Because we found relatively high incidences in Atlantic cod and sandeels caught in the pulse trawl (pulses-on), we subsequently zoomed in on these species separately. We assessed the effect of fish length on injury probability in pulses-on samples by adding the smoother for standard length as covariable in model (iii):

$$\text{(iii)} \qquad \mathrm{P}_{i,j} \sim Bin(1, p_{i,j}), logit(p_{i,j}) = \alpha + \beta_1 s(\mathrm{SL}_{i,j}),$$

where $\mathrm{P}_{i,j}$ corresponds to the proportion of spinal injuries per size class (*i*) in sampled trip (*j*), *s* is the non-linear smooth function, and SL is the fish standard length as also described in **Chapter 5**. We used a basis dimension *k* of four, which restricts the degrees of freedom of the smoother function and thus precludes overfitting. Spinal injury probability was predicted with 95% confidence intervals.

Finally, we quantified injury locations on the anteroposterior axis of the fish per spinal injury severity for pulses-on catches in Atlantic cod, lesser sandeel, and greater sandeel and tickler-chain samples for the latter two species. This allowed for a direct comparison to highly localised injuries in Atlantic cod exposed to electrical stimulation in the laboratory (de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c), and may reveal potentially different causes in wild caught Atlantic cod and sandeels.

All statistical analyses were performed in `R` v3.6.1 (R Core Team, 2019): generalized additive models were fitted using the `mgcv` package (Wood, 2021). Model fitted values and confidence intervals were calculated using the `predict` function in the `car` package (Fox and Weisberg, 2019) and back-transformed to the response scale with the inverse logit.

6.3 Results

In the 21 fishing trips, we collected 17,085 specimens of which 8,976 were sampled from pulses-on catches, 2,989 from pulses-off catches, and 5,120 from tickler-chain catches (**Table 6.2**). In total, we processed 26,176 X-ray images for sixteen fish species. We observed external discolouration marks on some, but not all Atlantic cod specimens with an injury.

6.3.1 Spinal injuries in pulses-on, pulses-off, and tickler-chain catches

Minor spinal injury incidences were relatively low, irrespective of catch method (**Table 6.2**). For pulses-on catches all incidences were lower than 5%, except for greater sandeel with an incidence of 13.6% and Atlantic cod (6.1%). For pulses-off catches insufficient numbers were collected for these species to enable comparisons. For tickler-chain catches we found, on average, slightly higher minor injury incidence levels, especially in flatfish species. Incidences for Atlantic cod were similar (6.8% and 6.1% for pulses-on and ticklers, respectively). For lesser sandeel, minor injury incidences were slightly higher in tickler-chain samples than in pulses-on samples, but for greater sandeel this pattern was reversed. We found no clear indications that such minor injuries were pulse related and due to low numbers of occurrences we refrain from a further statistical analysis. Instead, we focus on moderate and severe injuries, which previously have been associated with electrical pulsing. Because moderate spinal injuries were very rare (<1.5%), except for Atlantic cod caught with pulses on (14.5%), we grouped them in a single ‘major spinal injury’ class together with severe injuries.

For major injuries, we used the null model to estimate injuries probabilities per trip (**Figure 6.2**). Probabilities in pulse-trawl catches were low (<2%) in all of the 16 species

Table 6.2. Observed mean spinal injury probability per severity level for all species per catch method including number of sampled fishing trips and total number of animals collected. Species are grouped alphabetically by flatfish (common sole, dab, European plaice, and solenette), gadoids (Atlantic cod, bib, and whiting), gurnards (grey and tub gurnard), sandeels (lesser and greater sandeel), and others (bullrout, dragonet, European seabass, lesser weever, and striped red mullet).

Catch method	Species	Number of trips	Number of animals	Spinal injury probability per severity (*n*)					
				Minor		Moderate		Severe	
Pulses on	Common sole	6	824	2.9%	(24)	0.2%	(2)	0.4%	(3)
	Dab	4	765	3.8%	(29)	0%	(0)	0.1%	(1)
	European plaice	4	1684	1.7%	(28)	0.2%	(4)	0%	(0)
	Solenette	2	14	0%	(0)	0%	(0)	0%	(0)
	Atlantic cod	12	475	6.1%	(29)	14.5%	(69)	25.7%	(122)
	Bib	6	352	2.0%	(7)	0.3%	(1)	0.3%	(1)
	Whiting	12	2616	2.4%	(62)	0.2%	(5)	0.8%	(20)
	Grey gurnard	10	1071	1.8%	(19)	0.1%	(1)	0.3%	(3)
	Tub gurnard	7	200	4.0%	(8)	0%	(0)	2.0%	(4)
	Lesser sandeel	3	49	4.1%	(2)	0%	(0)	12.2%	(6)
	Greater sandeel	5	538	13.6%	(73)	1.5%	(8)	12.6%	(68)
	Bullrout	4	20	0%	(0)	0%	(0)	0%	(0)
	Dragonet	4	148	0.7%	(1)	0%	(0)	0%	(0)
	European seabass	3	103	1.0%	(1)	1.0%	(1)	0%	(0)
	Lesser weever	2	98	2.0%	(2)	0%	(0)	1.0%	(1)
	Striped red mullet	3	19	0%	(0)	0%	(0)	0%	(0)
Pulses off	Dab	3	636	3.8%	(24)	0.5%	(3)	0.2%	(1)
	European plaice	3	1631	2.6%	(43)	0.1%	(1)	0.2%	(4)
	Solenette	1	3	0%	(0)	0%	(0)	0%	(0)
	Atlantic cod	1	1	0%	(0)	0%	(0)	0%	(0)
	Whiting	3	586	2.0%	(12)	0%	(0)	0.9%	(5)
	Grey gurnard	2	116	0%	(0)	0%	(0)	0.9%	(1)
	Tub gurnard	1	16	0%	(0)	0%	(0)	0%	(0)
Tickler chains	Common sole	2	353	10.5%	(37)	0.6%	(2)	1.1%	(4)
	Dab	3	812	5.7%	(46)	0.2%	(2)	0.5%	(4)
	European plaice	3	1007	4.1%	(41)	0.3%	(3)	0.1%	(1)
	Solenette	2	8	12.5%	(1)	0%	(0)	0%	(0)
	Atlantic cod	4	103	6.8%	(7)	1.0%	(1)	0%	(0)
	Bib	3	4	0%	(0)	0%	(0)	0%	(0)
	Whiting	4	1148	3.0%	(35)	0.3%	(3)	2.4%	(27)
	Grey gurnard	3	1033	4.6%	(48)	0%	(0)	0.1%	(1)
	Tub gurnard	3	469	5.1%	(24)	0.6%	(3)	0.6%	(3)
	Lesser sandeel	2	112	8.0%	(9)	0.9%	(1)	25.9%	(29)
	Greater sandeel	2	33	6.1%	(2)	0%	(0)	39.4%	(13)
	Bullrout	1	1	0%	(0)	0%	(0)	0%	(0)
	Dragonet	2	27	0%	(0)	0%	(0)	0%	(0)
	Lesser weever	1	1	0%	(0)	0%	(0)	0%	(0)
	Striped red mullet	1	9	0%	(0)	0%	(0)	0%	(0)

studied, except for Atlantic cod and the two sandeel species. The injury probability of Atlantic cod retained in a pulse trawl was estimated at 41% (95% CI: 36–45%) which was significantly higher than the injury probability of Atlantic cod retained in a tickler-chain trawl (**Table 6.3**). For both sandeel species, significantly higher injury probabilities were observed for the animals caught in the tickler-chain trawl (on average 30%) as compared to the pulse trawl (on average 14%). Also, for whiting the injury probability was significantly higher in the tickler-chain trawl (2.6%) as compared to the pulse trawl (1%). Data for Atlantic cod and sandeels will be analysed in more detail in the next section. For an extended analysis of whiting data, we refer to **Chapter 5**. For the other species, injury probability did not differ significantly between pulse trawl and tickler-chain trawl caught fish (**Table 6.3**).

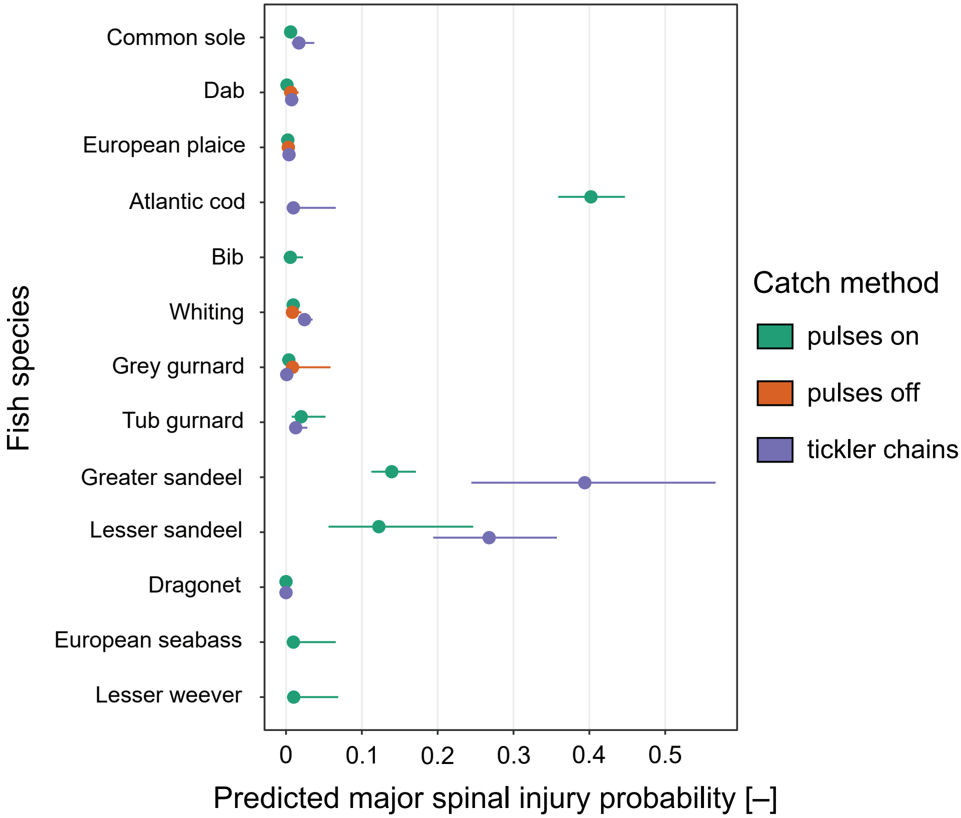

Figure 6.2. Spinal injury probability (plus 95% CI) for major injuries in species in pulses-on (green), pulses-off (orange), and tickler-chains (purple) catches. Injury probabilities were estimated by the null model. We only included species with a samples size of at least 90 specimens for one of the catch methods. For bib, European seabass, and lesser weever we can only show pulses-on estimates because we either had no tickler-chain samples or had found no injuries in these samples.

The effect of the pulse stimulus on the injury probability could be studied directly in four species (dab, grey gurnard, European plaice, and whiting) that were sampled in sufficient numbers from tows of a pulse trawl with the pulse stimulus switched off. The injury probability of these species is rather low and did not show a significant difference between the pulses-on and pulses-off catches (**Table 6.3**).

Table 6.3. Parameters estimates of the tickler-chains and pulses-off catch-method effect on the major injury rate in comparison with the pulses-on catch methods by fish species. Significant differences are indicated in bold.

Comparison	Species	Estimate	Standard error	*p*-value
Tickler-chains versus pulses-on	Common sole	1.0411	0.6089	0.0873
	Dab	1.7382	1.0813	0.1079
	European plaice	0.5158	0.7082	0.4664
	Atlantic cod	–4.2283	1.0092	**0.0000**
	Whiting	0.9520	0.2775	**0.0006**
	Grey gurnard	–1.3529	1.1189	0.2266
	Tub gurnard	–0.4541	0.6511	0.4855
	Lesser sandeel	0.9639	0.4852	**0.0470**
	Greater sandeel	1.3895	0.3774	**0.0002**
	Dragonet	0.7155	114801	1.0000
Pulses-off versus pulses-on	Dab	1.5760	1.1193	0.1591
	European plaice	0.2558	0.6717	0.7033
	Whiting	–0.1144	0.4920	0.8162
	Grey gurnard	0.8414	1.1223	0.4535

6.3.2 Spinal injuries in Atlantic cod

Atlantic cod clearly showed pulse-related major spinal injuries (**Figure 6.2**; **Table 6.2** and **6.3**). We therefore zoomed in on the prevalence of these injuries as function of fish length and their location on the anteroposterior axis.

Intermediate size classes, ranging between 20–40 cm, showed the highest major injury probability (**Figure 6.3A**). We found a significant effect of fish length on major spinal injury probability ($p < 0.01$) (**Figure 6.3B**). The locations of major injuries along the anterior-posterior axis show a narrow distribution at the posterior part of the abdominal region and anterior part of the caudal region (0.5–0.75) (**Figure 6.3C**). For minor spinal injuries in Atlantic cod, we found a broad distribution across the abdominal, caudal and ural regions.

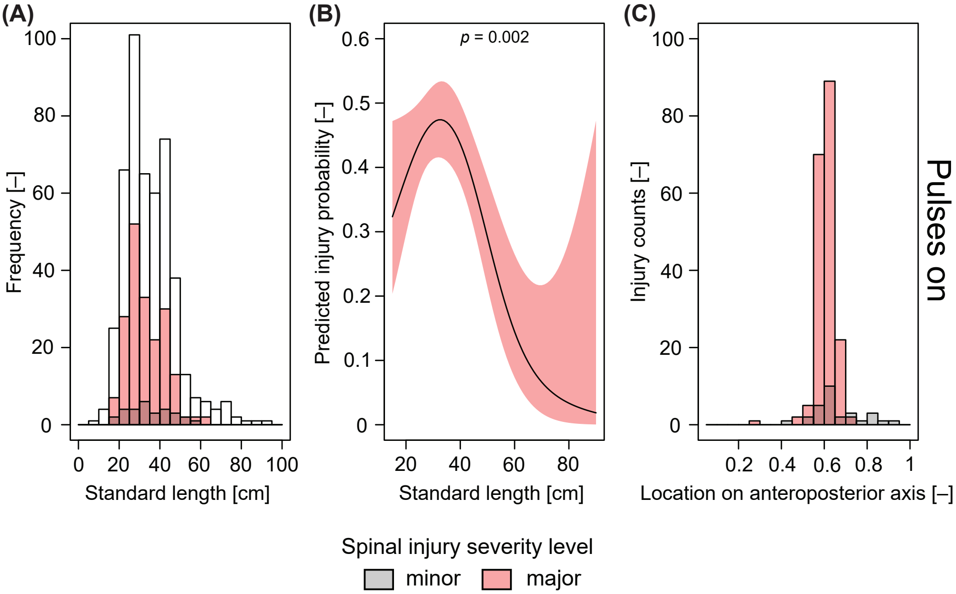

Figure 6.3. Spinal injuries in pulses-on catches of Atlantic cod. **(A)** Frequency distribution of minor (grey) and major (red) injuries and total number of fish analysed (white). **(B)** Predicted probability with 95% confidence interval of major spinal injuries from the generalized additive model. **(C)** Location of minor (grey) and major (red) injuries along the anteroposterior axis. Locations are defined as relative distances from snout (0) to caudal fin (1). Frequencies of minor and major injuries are overlayed and colours are semi-transparent, resulting in dark red for overlapping counts

6.3.3 Spinal injuries in sandeels

For sandeels, the probability of a major injury was higher in tickler-chain catches than in pulses-on catches. Fish length did not have a significant effect on the injury probability in lesser sandeel for both gears (**Figure 6.4B**, **E**) and for greater sandeel caught with tickler chains, but was significant in pulses-on samples for the latter species (**Figure 6.5B**, **E**). The spinal injury locations on the anteroposterior fish axis did not reveal a clear pattern that might indicate different, distinct causes for injuries between catch methods, in both species (**Figure 6.4** and **6.5**). Minor injuries tended to be located in the caudal regions whilst major injuries were located in the abdominal and caudal regions (0.30–0.75).

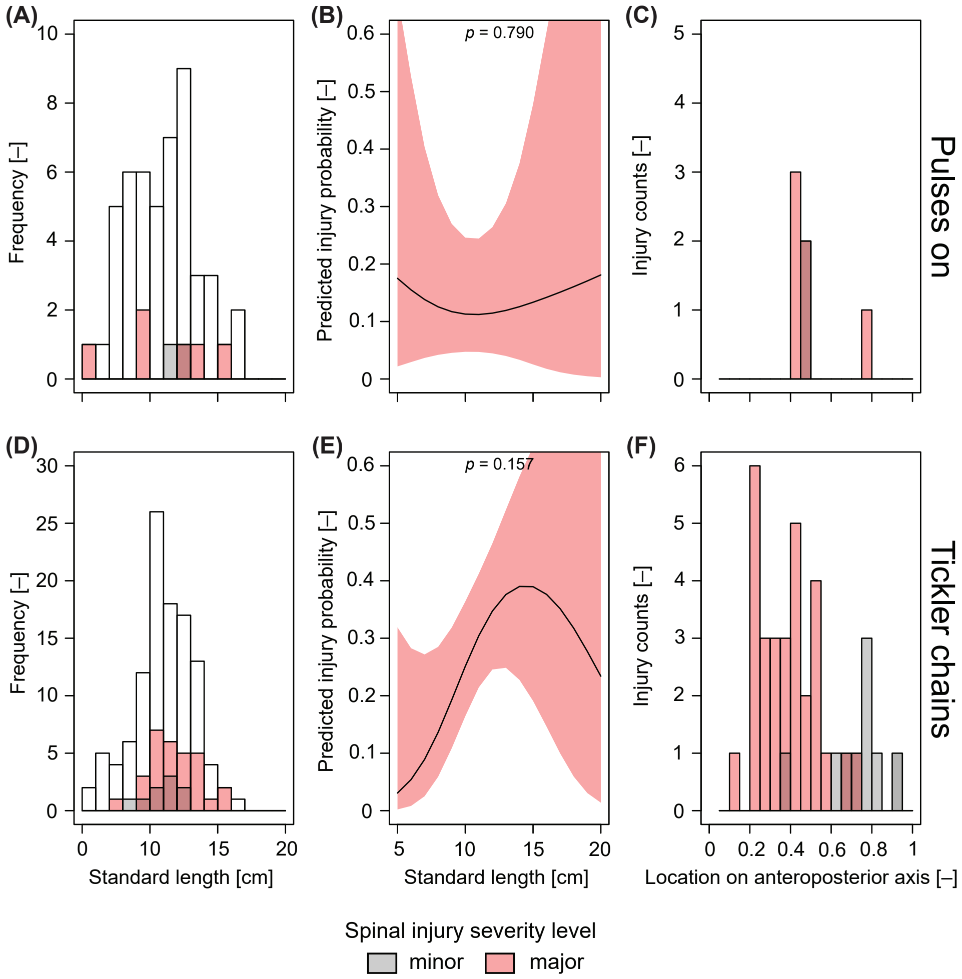

Figure 6.4. Spinal injuries in lesser sandeel caught in **(A–C)** pulse trawls and **(D–F)** tickler-chain beam trawls. **(A, D)** Frequency distribution of minor (grey) and major (red) injuries and total number of fish analysed (white). **(B, E)** Predicted probability with 95% confidence interval of major injuries from the generalized additive model. **(C, F)** Location of minor (grey) and major (red) injuries along the anteroposterior axis. Locations are defined as relative distances from snout (0) to caudal fin (1). Frequencies of minor and major injuries are overlayed and colours are semi-transparent, resulting in dark red for overlapping counts.

6.4 Discussion

The main question we addressed is to what extent electrical-pulse-induced spinal injuries occur in fish species that encounter pulse trawlers. Hereto, we assessed spinal injuries in sixteen fish species, target and non-target, round fish and flatfish as well as other body shapes, caught by pulse vessels with and without electrical stimulus

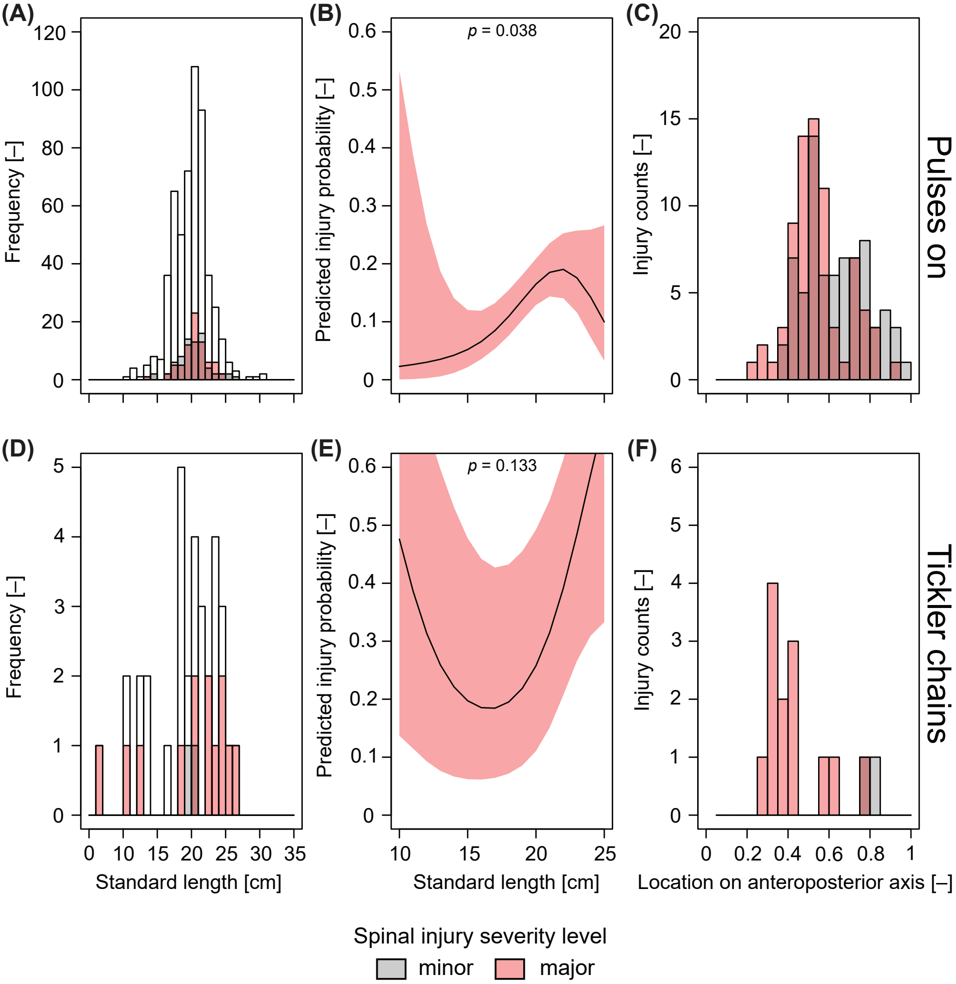

Figure 6.5. Spinal injuries in greater sandeel caught in **(A–C)** pulse trawls and **(D–F)** tickler-chain beam trawls. **(A, D)** Frequency distribution of minor (grey) and major (red) injuries and total number of fish analysed (white). **(B, E)** Predicted probability with 95% confidence interval of major injuries from the generalized additive model. **(C, F)** Location of minor (grey) and major (red) injuries along the anteroposterior axis. Locations are defined as relative distances from snout (0) to caudal fin (1). Frequencies of minor and major injuries are overlayed and colours are semi-transparent, resulting in dark red for overlapping counts.

and conventional tickler-chain beam trawlers. Although laboratory experiments are valuable in pinpointing the cause for internal injuries, they are impossible to conduct on a similar scale, and translation to field conditions can be problematic (Beaumont, 2016). By sampling under commercial circumstances, we provide direct information on catch method related injuries. Obviously, sampling on board of commercial vessels may introduce multiple biases related to e.g. weather conditions, fishing location, and time of year. For the comparison of pulses-on and pulses-off, we excluded these biases as much as possible by performing both settings on the same fishing trips. By sampling a relatively large number of fishing trips, we aimed to minimise potential differences between pulse and tickler-chain samples, and thus to provide a fair comparison. For comparing injuries with electrical pulse gears that had pulse stimulation either on or off, we gathered additional samples from cover nets surrounding the 80 mm mesh cod-ends. Without the cover nets we might have overlooked damages in small fish that escaped the cod-ends. For tickler-chain trips no cover net data were available. In comparing pulses-on samples to tickler-chain samples we should therefore realise that for tickler chains smaller specimens had a higher chance of escaping, which may have caused a bias towards higher injury incidences compared to pulse samples.

6.4.1 Spinal injuries in pulses-on, pulses-off, and tickler-chain catches

Our data show that spinal injuries were quite rare in 13 out of 16 species studied, both for pulse gears and tickler-chain gears. For bib, bullrout, common sole, dab, dragonet, European plaice, European seabass, grey gurnard, lesser weever, solenette, striped red mullet, tub gurnard, and whiting, we found minor and major injury rates below 12.5% and 2.6% respectively, and no indications for pulse-induced injuries as especially the latter category may be indicative of catch-method related injuries. Minor injuries could result from factors unrelated to catch method (**Chapter 5**). It should be noted that pulse and tickler-chain trawlers not only differ in the absence or presence of electrical stimulation, but also in the level of mechanical disturbance (Depestele *et al.*, 2016, 2019; Rijnsdorp *et al.*, 2020). Tickler-chain trawlers use the mechanical impact of heavy chains pulled through the sediment to startle fish, whereas pulse trawlers minimise mechanical disturbance by pulling electrodes strings in the direction of the trawl path. Moreover, pulse trawlers typically tow at lower speeds (**Table 6.1**) (van Marlen *et al.*, 2014; Poos *et al.*, 2020). Similar incidences of injuries in both catch methods, therefore, do not rule out injuries due to electrical stimulation. For all species, except sandeels and Atlantic cod, however, the sum of injuries that are potentially induced by electrical stimulation and by external mechanical impact, was low and not significantly different in pulses-on samples. Slightly higher incidences in tickler-chain samples might result from several sampling biases and are, therefore, inconclusive.

For common sole and European seabass, our findings match those of laboratory studies by Soetaert *et al.* (2016a, 2018) who reported absence of spinal injuries after electrical exposures of 146 and 31 specimens for each species respectively. Although we observed 5 out of 824 common sole with a major injury in pulses-on catches, a higher number of common sole was injured in the tickler-chain catches (6 out of 353), indicating that injuries were unlikely to be caused by electrical stimulation. No comparison with pulses-off was possible because common sole typically remain embedded in the sediment and will not bend in a U-shape (Soetaert *et al.*, 2016a; **Chapter 4**), which corroborates with Rijnsdorp *et al.* (2021b) who found higher footrope selection for common sole when switching on the electrical stimulus. We found one case (out of 103) of a major spinal injury in electrically-exposed European seabass, but data for a comparison to pulses-off or tickler-chain gears was missing. For the remainder of species for which we found low injury rates, to our knowledge, no spinal injury data is available in the literature for common-sole-targeting pulse trawling. After exposures to 5 Hz pulsed electrical stimulation for targeting brown shrimp (*Crangon crangon*), Desender *et al.* (2016) found no spinal injuries in armed bullhead (*Agonus cataphractus*), Atlantic cod, bullrout, common sole, and European plaice. This low-frequency stimulation, however, does not induce whole-body muscle contractions (i.e. referred to as muscle cramp, or tetanus,) which are hypothesised to cause pulse related injuries in marine electrotrawling (e.g. Soetaert *et al.*, 2019). This would correspond to the finding that in freshwater electrofishing, spinal injury incidence was found to increase with pulse frequency (Dolan *et al.*, 2002; Snyder, 2003; Reynolds and Dean, 2020). Sharber *et al.* (1994) hypothesised that spinal injuries were caused by myoclonic jerks associated with shock-induced seizures, and that such seizures occur more frequently at higher stimulation frequencies. Waveforms and exposure circumstances in freshwater electrofishing are, however, different and cannot be directly compared to marine electrotrawling.

For three species, Atlantic cod, lesser sandeel, and greater sandeel, we found substantial injury incidences that warrant further discussion. In Atlantic cod, 40.2% showed a major spinal injury in pulses-on samples ($n = 475$) whereas in tickler-chain samples this was only 1% ($n = 103$). This partly corroborates previous field and laboratory experiments that showed spinal injuries due to electrical pulsing in Atlantic cod (van Marlen *et al.*, 2014; de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c, 2016d). While the sensitivity of Atlantic cod to pulse-induced spinal injuries has been well-documented, injury incidences in well-controlled laboratory exposure studies have varied widely, with sometimes substantially lower incidences (Soetaert *et al.*, 2016c). It remains unclear why injury rates in laboratory exposure experiments are lower than for fish sampled on board of commercial vessels. It might be related to swimming behaviour of Atlantic cod during the catch process. They typically enter low in trawl nets, staying close to the seafloor (Main and Sangster, 1985; Krag *et al.*, 2010), where electric field strengths are highest close to the electrode arrays (e.g. **Chapter 4**). If Atlantic cod would swim close to the electrodes, they would always be exposed to the highest possible electric field strengths, which would be well above the value of 80 V m^{-1} that according to de Haan *et al*. (2016) gives a 50% chance for spinal injuries (95% CI: 60–110 V m^{-1}). The field study by van Marlen *et al*. (2014) reported a lower injury incidence in Atlantic cod (7.4% and 11.1% in two fishing trips), but their numbers were based on a small sample size ($n = 27$ and $n = 18$) and damages were scored without X-radiographs, which is less effective in detecting spinal injuries (McMichael, 1993). Moreover, fish sizes studied were relatively large (55.0 ± 15.9 cm and 48.7 ± 16.2 per trip) whereas injured specimens were generally small at 20, 23, 27, and 55 cm (for photographs, see van Marlen *et al*., 2011). This matches our finding that Atlantic cod between 20–40 cm are more prone to major spinal injuries compared to smaller or larger specimens. De Haan *et al*. (2016) also reported that small (≤17 cm) and large specimens (≥50) were less susceptible to spinal injuries in laboratory exposures. The size effect may explain why Soetaert *et al*. (2016b) found variable injury incidences in the laboratory as they used relatively large fish (most being >40 cm TL). We suggest that future studies on pulse-injuries in Atlantic cod should always use X-radiography, assess the effect of fish length on the spinal injury incidence, and state whether standard length or total length is used.

In addition to high injury rates in Atlantic cod, which had been reported previously, we found high major injury incidences in pulses-on catches of lesser and greater sandeel (12.6% and 14.1% respectively). Both species, however showed significantly higher incidences in the tickler-chain samples (26.8% and 39.4%), indicating that external mechanical impact might be the main cause for these injuries. Injury locations on the anteroposterior axis also revealed no clear pattern that might indicate different, distinct causes for injuries between catch methods. Further evidence that the observed injuries in our study were more likely caused by mechanical impact during the catch process was provided by laboratory exposures of sandeels that did not reveal any pulse-induced injuries (ICES, 2020b). Susceptibility to mechanical impact may very well be related to their long, slender body form and their tendency to dig into the sediment. This would make sandeels vulnerable to the heavy tickler chains that are dragged through the sediment at high speed. Electrode arrays of pulse gears are dragged in the lengthwise direction and typically penetrate the sediment less deep (Depestele *et al*., 2019). Moreover, the long slender shape of sandeels might also make them more vulnerable to damage from the nets. We cannot, however, exclude that a selection bias for injured specimens also played a role. Sandeels can easily escape the nets used in common sole fisheries. For reference, fisheries targeting sandeels use mesh sizes smaller than 16 mm (European Parliament and Council, 2019), which is much smaller than mesh sizes in common-sole-targeting gears. If injured specimens are less likely to escape the nets, we may have overestimated the percentage of fish with injuries. Such selection bias may have affected the comparison between catch methods, because part of the sandeels from pulse gears were collected in cover nets. Although the meshes of the cover nets allow sandeels to escape, we cannot exclude that a higher percentage of non-injured sandeels was retained in the cover nets. However, because electrical-pulse exposures of sandeels in laboratory conditions show that these species are insusceptible to pulse-induced injuries (ICES, 2020b), mechanical trauma presumably plays a major role. The use of tickler chains, higher towing speeds for tickler-chain gears, and substantially higher total catch weights are likely to contribute to higher injury rates in tickler-chain gears than in pulse gears. In whiting, we also saw increased injury rates in tickler-chain samples, which may also indicate that potential injuries from pulse exposures are outweighed by mechanically-induced injuries.

It remains unclear why, of all species studied, only Atlantic cod seems vulnerable to electrical pulsing. Morphological and anatomical differences (e.g. body length and shape) as well as fish behaviour may cause sensitivity differences for pulse-induced spinal injuries. Scale type and vertebra size and number have been postulated to explain interspecies differences, similar to findings in freshwater electrofishing (Soetaert *et al.*, 2018). In our study, major spinal injuries where highly localised at the posterior abdominal and anterior caudal region, which corroborates findings for Atlantic cod exposed in the laboratory (de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c). Localised injuries may be related to several factors, e.g., body shape, regional variations in muscle distributions, and regional variations in vertebrae morphology. Fjelldal *et al.* (2013) described for Atlantic cod that the length of vertebrae in the cervical and abdominal regions increases with age, whilst length decreases in the caudal and ural regions. Furthermore, they reported, for an adult specimen, that the vertebral column is evenly flexible (left-right bending) apart from the ural regions, but do not take intervertebral ligaments into account. In Atlantic salmon, the largest vertebrae contain the highest mineral content and are located where the imposed mechanical load is highest during normal swimming (Fjelldal *et al.*, 2005, 2006). For whole-body muscle cramps during electrical stimulation this might cause localised spinal injuries. For whiting, also a gadoid, we did not find a similar strict localisation of spinal injuries (**Chapter 5**). Rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), saithe (*Pollachius virens*), and Atlantic herring (*Clupea harengus*) have shown to be prone to electrically-induced injuries, albeit with a different stimulus (continuous, 50 Hz modulations for electrostunning), which may be related to having many, relatively small vertebrae, in contrast to, e.g., European seabass which has relatively large and few vertebrae and is not prone to spinal injuries (Snyder, 2003; Roth *et al.*, 2004; Nordgreen *et al.*, 2008; de Haan *et al.*, 2016; Soetaert *et al.*, 2016b, 2016c, 2018). In flatfish, the asymmetry of muscle mass on either side of the body may protect the spinal column from excessive overload from two-sided contractions typically resulting from pulse stimulation. Other differences, however, may also play a role and additional studies on functional morphology of muscles, ligaments, and skeleton are required to pinpoint the causes for absence of presence of spinal injuries and their localisation.

6.4.2 Ecological effects of pulse trawling

Advantages of pulse trawling over traditional tickler-chain trawling have been discussed previously. A potential main disadvantage is pulse-induced spinal injuries. Here, we studied the incidence of such injuries in a wide range of species, to assess the associated negative ecological impact. Our results show that injuries, whether pulse induced or from mechanical trauma, are rare in most of the species studied. For these species, our study reveals no negative effects of pulse trawling that would counteract the benefits. Some of the benefits are well documented and would therefore favour electrical-pulse trawling over tickler-chain fishing. Flatfish, for example, are not susceptible for pulse-induced injuries and have been shown to have higher discard survival rates (van der Reijden *et al.*, 2017). For species with a swim bladder, discard survival rates are less relevant because they mostly suffer from barotrauma irrespective of catch method (Davis, 2002; Benoît *et al.*, 2013; Depestele *et al.*, 2014). For these species, the escape probability of small specimens is presumably the dominant factor determining the ecological impact. There are no indications that escaping from pulse gears would be hampered and lower towing speeds in combination with lower net loads would actually favour escape probability. In lesser and greater sandeel, we found substantial injury probabilities, but found no evidence that they were caused by electrical pulsing.

For Atlantic cod we did find high incidences of pulse-induced injuries that potentially affect their populations. Here, the effects on small specimens that may escape the nets are especially relevant. Fish retained in the nets are either landed, or discarded with low survival probability (Lindeboom and de Groot, 1998; Depestele *et al.*, 2014). Minimum landing size is 35 cm (total length) in the North Sea (Council of the European Union, 1998; European Parliament and Council, 2019) and 80 mm meshes have a 50% retention probability at about 18 cm (Reeves *et al.*, 1992). Although we sampled relatively few small Atlantic cod, our data suggests that specimens sufficiently small to escape through the meshes might be less susceptible to electrical-pulse-induced spinal injuries. A similar finding was reported by de Haan *et al.* (2016), who also noted that these fish resumed normal behaviour after exposure. Overall injury rates for Atlantic cod therefore presumably overestimate the effects for the more relevant, smaller specimens. We recommend a modelling-based upscaling assessment to explore the potential additional mortality imposed by spinal injuries in small specimens on Atlantic cod populations in the North Sea.

In conclusion, we found low spinal injury probabilities in the majority of species irrespective of catch method, apart from Atlantic cod, lesser sandeel, and greater sandeel. For sandeels, spinal injuries were most likely related to mechanical impact rather than to electrical stimulation. Our results confirm pulse-induced spinal injuries in Atlantic cod. This effect, however, seems to be highly Atlantic-cod-specific and relatively less severe in smaller specimens.

6.5 Statements

6.5.1 Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6.5.2 Author contributions

All authors contributed to the conception and design of the study; PGB, WSMV and RPMP processed the dead fish; MJL programmed the database program; PGB and WSMV collected the data from the X-ray images. PGB, ADR, and WSMV performed the statistical analyses. All authors interpreted the data and discussed the results; PGB drafted the initial manuscript and figures, with contributions by ADR, JLvL, WSMV, RPMP, and MJL. All authors contributed to the critical revision of the manuscript and figures, and approved the final version.

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Supplementary material

Supplementary material 6.1 Details on sampling per species with number of animals per trip and net part.

Table S6.1A. Trawler sampling overview of lesser sandeel (*Ammodytes tobianus*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V1	2016	29	Pulses on	0	1
V5	2017	24		0	16
		33		0	32
V10	2018	23	Tickler chains	26	0
		26		86	0

Table S6.1B. Trawler sampling overview of bib (*Trisopterus luscus*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V2	2018	4	Pulses on	4	0
V3	2017	6		1	0
V5		24		1	0
V6		44		177	0
V7	2018	4		5	0
V9		8		164	0
V10		26	Tickler chains	2	0
V11		47		1	0
	2019	9		1	0

Table S6.1C. Trawler sampling overview of European seabass (*Dicentrarchus labrax*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V6	2017	44	Pulses on	8	0
V7	2018	4		1	0
	2019	5		94	0

Table S6.1D. Trawler sampling overview of Atlantic cod (*Gadus morhua*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	**Cover**
V2	2016	41	Pulses on	3	0
	2017	36		11	0
	2018	4		1	0
			Pulses off	22	0
		46	Pulses on	64	0
V4	2017	7		116	0
V5		24		47	0
V6				1	0
		44		61	0
V7	2018	4		32	0
	2019	5		18	0
V8	2018	6		52	0
V9		8		48	0
V10		26	Tickler chains	3	0
V11		47		24	0
	2019	9		50	0
V12		8		26	0

Table S6.1E. Trawler sampling overview of dab (*Limanda limanda*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	**Cover**
V1	2016	29	Pulses on	140	0
			Pulses off	192	0
V2	2018	4	Pulses off	4	0
V5	2017	24	Pulses on	0	539
		33		0	84
V9	2018	8		2	0
			Pulses off	440	0
V10		23	Tickler chains	244	0
		26		567	0
V11		47		1	0

Table S6.1F. Trawler sampling overview of solenette (*Buglossidium luteum*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	**Cover**
V2	2018	4	Pulses off	3	0
V5	2017	24	Pulses on	0	6
		33		0	8
V10	2018	23	Tickler chains	6	0
		26		2	0

Table S6.1G. Trawler sampling overview of grey gurnard (*Eutrigla gurnardus*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V1	2016	29	Pulses on	60	0
			Pulses off	62	0
V2	2018	4	Pulses on	34	0
			Pulses off	56	0
		36	Pulses on	94	0
V4	2017	7		37	0
V5		24		0	84
		33		0	350
V6		24		401	0
V7	2018	4		2	0
V8		6		6	0
V9		8		1	0
V10		23	Tickler chains	308	0
		26		340	0
V11		47		385	0

Table S6.1H. Trawler sampling overview of tub gurnard (*Chelidonichthys lucerna*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V1	2016	29	Pulses on	23	0
			Pulses off	16	0
V2	2018	4	Pulses on	6	0
		36		13	0
V5	2017	24		0	20
		33		0	12
V6		24		125	0
		44		1	0
V10	2018	23	Tickler chains	174	0
		26		286	0
V11		47		9	0

Table S6.1I. Trawler sampling overview of dragonet (*Callionymus lyra*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V1	2016	29	Pulses on	0	34
V5	2017	24		0	93
V6				19	0
V8	2018	6		2	0
V10		23	Tickler chains	6	0
V11		47		21	0

Table S6.1J. Trawler sampling overview of striped red mullet (*Mullus surmuletus*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V1	2016	29	Pulses on	0	1
V5	2017	24		0	12
		33		0	6
V11	2018	47	Tickler chains	9	0

Table S6.1K. Trawler sampling overview of bullrout (*Myoxocephalus scorpius*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V1	2016	29	Pulses on	0	11
V5	2017	33		0	1
V6		24		7	0
V8	2018	6		1	0
V10	2018	23	Tickler chains	1	0

Table S6.1L. Trawler sampling overview of European plaice (*Pleuronectes platessa*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V1	2016	29	Pulses on	0	175
			Pulses off	59	0
V2	2018	4		829	0
V5	2017	24	Pulses on	0	752
		33		0	755
V9	2018	8		2	0
			Pulses off	743	0
V10		23	Tickler chains	458	0
		26		518	0
V11		47		31	0

Table S6.1M. Trawler sampling overview of common sole (*Solea solea*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V1	2016	29	Pulses on	96	0
V2	2018	4		2	0
V4	2017	7		1	0
V5		24		0	453
		33		0	271
V9	2018	8		1	0
V10		23	Tickler chains	107	0
		26		246	0

Table S6.1N. Trawler sampling overview of lesser weever (*Echiichthys vipera*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V1	2016	29	Pulses on	0	97
V5	2017	33		0	1
V10	2018	26	Tickler chains	1	0

Table S6.1O. Trawler sampling overview of whiting (*Merlangius merlangus*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V1	2016	29	Pulses on	48	333
			Pulses off	67	198
V2		41	Pulses on	563	0
	2018	4		40	0
			Pulses off	186	0
V3	2017	6	Pulses on	125	0
V4		7		85	0
V5		24		0	609
		33		0	275
V6		24		6	0
		44		149	0
V7	2018	4		198	0
V8		6		117	0
V9		8		68	0
			Pulses off	135	0
V10		23	Tickler chains	93	0
		26		9	0
V11		47		795	0
	2019	9		251	0

Table S6.1P. Trawler sampling overview of greater sandeel (*Hyperoplus lanceolatus*) per vessel (anonymised coding) and trip with catch year and week, catch method, and number of collected animals per net part.

Vessel	Year	Week	Catch method	Number of animals	
				Cod-end	Cover
V1	2016	29	Pulses on	0	26
V5	2017	24		0	284
		33		0	206
V6		24		14	0
		44		8	0
V10	2018	23	Tickler chains	9	0
		26		24	0

Chapter 7

General discussion

In this thesis, we aimed to contribute to understanding the effects of electrical stimulation on marine organisms. Furthermore, we aimed to provide input for assessing the impact of flatfish electrotrawling on the marine ecosystem. The study of animal responses to pulsed electric fields is challenging because of complex interactions of organisms with the electric field and the large amount of response variables that could be assessed for an equally large range of species, both in laboratory and field conditions. The potential of electricity to capture, anaesthetise, and kill aquatic organisms has fascinated humans for centuries (Baggs, 1863; de Groot and Boonstra, 1974) and has led to applications in fish research, freshwater fisheries management, and commercial marine electrotrawls (Soetaert *et al.*, 2015a; Beaumont, 2016; Reid *et al.*, 2019; Reynolds and Dean, 2020). Historically, most marine studies had focused on fuel consumption, catch efficiency, and selectivity of pulse trawls as well as overcoming technical challenges that arise when electrofishing in highly conductive water (**Chapter 1**). From the 2000s onwards, focus shifted to the effects of electric fields on organisms and the environmental impact (Polet, 2003; Soetaert, 2015; Desender, 2018; Tiano, 2020). However, quantitative information on behavioural responses of benthic invertebrates and fishes is lacking as well as quantitative data on the incidence of internal injuries in fish species that encounter pulse trawls. Knowledge on these topics will shed more light on potential effects of pulse trawling on marine organisms and impact on the ecosystem.

To increase our understanding of electrical pulsing on marine organisms, we studied behavioural responses, survival, and internal injuries in a range of benthic invertebrates and fish species. In **Chapter 2**, we concisely review the marine electrotrawling field, describe physiological responses of organisms exposed to electrical stimulation, and outline waveform characteristics to hopefully facilitate communication and promote collaboration with freshwater electrofishing researchers. We quantified behavioural responses and survival the benthic invertebrates common starfish (*Asterias rubens*), serpent star (*Ophiura ophiura*), common whelk (*Buccinum undatum*), sea mouse (*Aphrodita aculeata*), common hermit crab (*Pagurus bernhardus*), and flying crab (*Liocarcinus holsatus*). We studied species-specific locomotion behaviours before, during, and after electrical stimulation and for a control group and monitored 14–days survival afterwards (**Chapter 3**). Concerns had been expressed regarding the sensitivity of electroreceptive fish species and fishes in general (Desender *et al.*, 2017a; ICES, 2018; Quirijns *et al.*, 2018). In **Chapter 4**, we investigated the behavioural response thresholds of the electroreceptive small-spotted catshark (*Scyliorhinus canicula*) and thornback ray (*Raja clavata*), and the non-electroreceptive European seabass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*), and common sole (*Solea solea*) for electric field strengths. We subsequently related these – with the help of numerical simulation – to the electric field around commercial electrode arrays. Apart from behaviour and survival, the uncertainty on fish-species susceptibility to electrical-pulse-induced internal injuries was another concern after observations of injuries in Atlantic cod (*Gadus morhua*) and whiting (*Merlangius merlangus*) (van Marlen *et al.*, 2014; de Haan *et al.*, 2016; Soetaert *et al.*, 2016a, 2016b, 2016c). To address these concerns, in **Chapter 5**, we examined spinal injuries and internal haemorrhages in whiting caught with pulses-on and pulses-off by commercial electrotrawls and specimens caught by conventional tickler-chain trawls. We assessed the injury incidence as function of fish-length, injury type co-occurrence, and location of the injuries on the anteroposterior body axis for different severity categories. Finally, we assessed spinal injuries in a similar manner for sixteen fish species caught according to the same protocol, i.e. in Atlantic cod, bullrout (*Myoxocephalus scorpius*), common sole, dab (*Limanda limanda*), dragonet (*Callionymus lyra*), European plaice (*Pleuronectes platessa*), European seabass, greater sandeel (*Hyperoplus lanceolatus*), grey gurnard (*Eutrigla gurnardus*), lesser sandeel (*Ammodytes tobianus*), lesser weever (*Echiichthys vipera*), bib (*Trisopterus luscus*), solenette (*Buglossidium luteum*), striped red mullet (*Mullus surmuletus*), tub gurnard (*Chelidonichthys lucerna*), and whiting (**Chapter 6**).

In the following discussion, we place our findings presented in this thesis into a wider context. In section 7.1, we integrate most important results of research chapters with existing literature on effects of electric fields on (marine) organisms. In addition, we discuss the limitations of our experimental setups and approaches as well as some unaddressed topics in the thesis. In section 7.2, we explore the biomimetic potential of electroreceptive and electrogenic fish species for human (electro)fishing techniques. In particular, we provide an outlook on the design of novel electrical detection and stimulation possibilities. In section 7.3, we present future research perspectives with numerical simulation and fishing gear innovation. Finally, we conclude this thesis by returning to the motivation of our research, namely the sustainable harvest of marine fish stocks with minimal environmental impact. We touch upon the societal relevance of our findings and how we envision future fishing gear innovation (section 7.4).

7.1 Effects of electric fields on marine organisms

Electrofishing is a broad term that encompasses the application of an electric field in the water to incapacitate fish or invertebrates, rendering them easier to control or capture, either by stimulating the organism to move towards the fishing gear or immobilisation (Reynolds and Kolz, 2012; Beaumont, 2016). To understand the effects of electrofishing, knowledge is required on the underlying principles and mechanisms that determine the response of marine organisms in an electric field. Historically, research has focused on the physiological and behavioural responses of various marine animals (**Chapter 1**). Specifically, researchers were interested in applying knowledge from freshwater electrofishing to marine species. During the past century, various theories have been developed to explain the specific responses of aquatic organisms to electric fields, and how to utilise these responses in capture fisheries. In the following sections, we will concisely address the postulated underlying principles and mechanisms of how electric fields affect marine animals. This background knowledge provides a reference frame on how to interpret our findings in the subsequent sections on benthic invertebrates and fishes.

7.1.1 Action mechanism of the electric field

The exact nature of the action mechanism of electric fields on aquatic organisms is still under discussion (Sternin *et al.*, 1976; Reynolds *et al.*, 1988; Sharber and Black, 1999; Snyder, 2003; Reynolds and Kolz, 2012; Beaumont, 2016). The responses of fish to an electric field have been described as function of distance from the electrodes, with increasing response activity in higher electric field strengths closer to the electrodes (Snyder, 2003; Polet, 2010). The observations can be summarised into five main responses, each occurs with decreasing distance to the electrodes: (i) 'fright' or 'minimal response' is the initial startle response when a detection threshold is reached; (ii) 'galvanotaxis'/'electrotaxis' is induced directed swimming or movement, typically oriented towards a specific electrode if (pulsed) direct current is used (for fish mostly the anode); (iii) 'electronarcosis' is immobilisation of fish through electroanaesthesia; (iv) 'electrotetanus' is the immobilisation of fish through induction of muscle contractions and may be accompanied with epileptic seizures; (v) death. More recently, Reid *et al.* (2019) recommended that 'electroimmobilisation' should be used as umbrella term to cover all forms of reversible sleep-like states such as electosedation, electroaneasthesia, electrotetany, and electrostunning. Fish responses may differ depending on the type of waveform, species, and size (Vibert, 1967; Sternin *et al.*, 1976; Dolan and Miranda, 2003; Snyder, 2003; Polet, 2010; Beaumont, 2016)

Three postulated underlying principles which determine the response of a fish (e.g. twitch, electrotaxis, and electronarcosis) are the 'Biarritz Paradigm', 'Power Transfer Theory', and the 'Bozeman Paradigm' (Snyder, 2003; Beaumont, 2016). The Biarritz Paradigm considers that the central nervous system, peripheral nervous system, and the fish's muscles respond directly to the electrical stimulus and suggests that fish behaviour is a summation of local cellular responses of both neurons and muscle fibres. This view was hypothesised based on a series of papers of collaborating scientists at the Biarritz Hydrobiological Station in France (Vibert, 1963; Blancheteau, 1967; Lamarque, 1967, 1990) and contains underling principles of Kolz's Power Transfer Theory (PTT). Kolz (1989) and Kolz and Reynolds (1989) hypothesised that fish muscles respond to electric fields via neural responses and that these responses are induced by electrical power levels in the organism. The electrical stimulus should be of sufficient power to interact with neurons, and fish responses are directly related to the magnitude of the power density transferred from the water to the fish (a parameter that integrates voltage gradient and current density) (Miranda and Dolan, 2003). The Bozeman Paradigm, proposed by Sharber and Black (1999), specifies that the observed fish responses are similar to stages of electrically-induced epilepsy (e.g. automatism, petit mal, grand mal) and suggests that fish behaviour is a neurological reaction to overstimulation of the central nervous system (Sharber *et al.*, 1994). The specific role of the fish's nervous system and muscles in determining the response in an electric field has been subject of numerous studies. For example, Danyulite and Malyukina (1967) studied the effect of a DC electric field on, amongst others, unanaesthetised Baltic herring (*Clupea harengus membras*) by either blocking skin receptor function, removing the skin, removing parts of the brain, or destructing the spinal cord (translation to Dutch or English via: de Groot and Boonstra, 1974; Sternin *et al.*, 1976). They found that the spinal cord is required for galvanotaxis as this response occurred in all instances except when the spinal cord was disconnected. This matches findings that fish swimming is coordinated by interneurons in the spinal cord (**Chapter 2**). Given the variety of research findings on effects of electric fields and potential interactions with waveform, body size, species, and orientation in the electric field, no conclusive explanation can be provided for the underlying principles of fish responses (Snyder, 2003; Beaumont, 2016). We will use aforementioned principles to interpret our findings.

The electrode arrays of marine electrotrawls generate heterogeneous, (pulsed) electric fields between the electrodes which affect the organisms around them for subsequent capture (here we include the razor-clam electrofishing gear as 'trawl') (**Chapter 1** and **2**). Depending on the target species (i.e. trawl type) the electrical stimulus: (i) induces muscle contractions that immobilise fish lasting for the duration of exposure ('common sole pulse'); (ii) induces muscle contractions that, due to the lower frequency, result in twitch-like responses ('brown shrimp pulse'); (iii) induces escape-like movements whereby razor clams (*Ensis* spp.) emerge from the sediment within one minute and 'kick' their muscular foot ('razor clam waveform') (Polet *et al.*, 2005; Soetaert *et al.*, 2015a, 2015b; Desender *et al.*, 2016; Murray *et al.*, 2016).

Electric fields generated by electrotrawls have been studied in experimental setups and in the field with in situ measurements and computer simulations (**Figure 4.1**, **4.3**) (Polet *et al.*, 2005; Verschueren and Polet, 2009; de Haan *et al.*, 2016; Murray *et al.*, 2016; Soetaert *et al.*, 2016a; de Haan and Burggraaf, 2018). Electric field strengths around commercial electrode arrays are always highest close to the electrodes and diminish nonlinearly and rapidly with distance from the electrodes (**Figure 4.3**, **4.5**). Pulse trawls generate the electric field with a constant power output throughout the year but pulse amplitude at the seafloor drops with 2–3 volts in the summer months (ICES, 2020a). A general misconception is that water conductivity affects the voltage gradient (Reynolds and Kolz, 2012; ICES, 2020a). Voltage gradient distribution maps are, however, largely independent of water and sediment conductivity in a homogeneous environment and scale linearly with applied voltage amplitude (**Chapter 4**). Whereas field strengths are largely independent of the medium's conductivity, higher conductivities allow for higher currents and may thus affect organisms. Hence, to assess effects of electric fields on organisms, we need to take the discrepancy of conductivity between the animal and surrounding media into account, as also demonstrated by Soetaert *et al.* (2016a). Here, knowledge is required on the internal electric fields in the fish, because muscle or neural activity thresholds are determined by local electric field strengths inside the animal. As discussed in the previous paragraph, involuntary muscle contractions occur when internal thresholds of neurons and/or muscles for electrical stimulation are exceeded.

To explore and estimate susceptibility of fish to electric fields, field strengths inside a model fish were estimated by placing idealised shapes into the COMSOL model presented in **Chapter 4** (**Figure 7.1**) (COMSOL Multiphysics®, n.d.). Internal electric field strengths differed from the surrounding external fields, due to conductivity differences of the organism's body relative to the seawater when fish were placed at the same position and orientation in the water column and sediment with respect to the electrode pair (**Figure 7.1A**). We subsequently replaced the round fish model in the sediment with a flatfish (**Figure 7.1B**). Mean internal field strengths were extracted from the **(C)** round-fish and **(D)** flatfish model as function of the horizontal distance to the centre of the electrode pair for different heights in the water column and depths in the sediment respectively. Although external electric fields were similar in the water column and sediment, the buried flatfish was somewhat protected in the sediment from high internal field strength compared to the round fish (note the difference in step size between the height in the water column and depth in the sediment). Mean internal fields strengths in both fish types steeply decline with height and depth, and even more steeply as a function of distance to the electrode. Peak stimulations occur, in both cases, when fish are immediately above or below an electrode. Although it remains uncertain

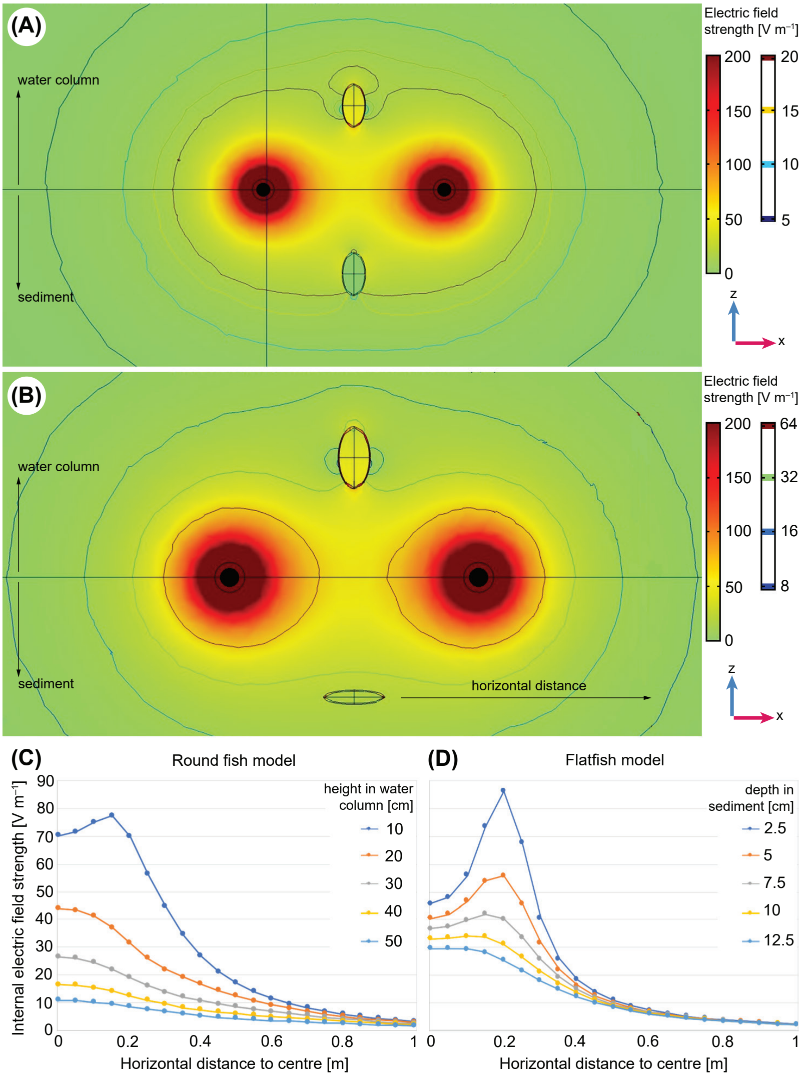

Figure 7.1. Simulations of electric field strengths around an electrode array pair (black dots) and in a fish. The simulation is similar as described in **Chapter 4** but with a simplified fish situated between the electrodes, in the water column and sediment. Fish were simulated as ellipsoids, with 2 mm thick skin and a body (30 cm long), with a conductivity of 0.1 and 0.5 S m^{-1} respectively. Water and sediment conductivity were set to 5 and 0.5 S m^{-1} respectively. Electrode arrays were 41.5 cm apart and electrodes were 12 cm long and 3 cm in diameter, separated by 22 cm of insulated parts. Field strength values close to the electrodes are clipped at 200 V m^{-1}. Contour lines indicate equal field strengths (thin lines from blue to brown). **(A)** Vertical cross-section through the centre of the round fish in the water column and sediment, orthogonal to the electrode pair. **(B)** Vertical cross-section through the centre of the round fish in the water column and flatfish in the sediment, orthogonal to the electrode pair. Panel is zoomed in with respect to **(A)** and data extracted from this simulation were used for **(C)** and **(D)**. **(C)** Mean internal electric field strength in the round fish model as function of horizontal distance to the centre of an electrode pair for different heights in the water column. **(D)** Mean internal electric field strength in the flatfish model as function of horizontal distance to the centre of an electrode pair for different heights in the water column. Data and figures were partly presented in ICES (2020a).

whether, e.g., the mean or maximum internal electric field strength in a fish explains the onset of a response, the response will be local because we observed muscle twitches in only those parts of a fish's body closest to a small electrode pair whilst other body parts did not show signs of muscle twitching (ICES, 2020a). Based on our simulations, flatfish buried in the sediment, and possibly other organisms as well, experience relatively lower electric field strengths inside their body. This interaction shows that the electric fields of pulse trawls to stimulate flatfish from the sediment are relatively unfavourable for fish in the water column. Nonetheless, the effects are highly local as internal electric fields, irrespective for round fish and flatfish, drop below a value of about 20 V m^{-1} at a horizonal distance of 50 cm from the electrode-pair centre and is hardly affected by positions in the vertical plane.

7.1.2 Marine benthic invertebrates

Understanding the physiological effect of electric fields on marine invertebrates is a rather unexplored research field. Preceding electrofishing theories have, to our knowledge, only been applied in fish, and mainly in freshwater. For marine invertebrates, few physiological literature reports are available in the context of electrofishing. In the late 19th century and early 20th century, galvanotaxis was studied in a range of echinoderm species (Nagel, 1893; Scheminzky, 1931a, 1931b). However, many early papers, also those focusing on fish (e.g. in Russian or Chinese), were not always translated and rarely cited (Sternin *et al.*, 1976; Yu *et al.*, 2007; ICES, 2010, 2011), which might explain loss of insights and knowledge.

Because previous studies were rare and no detrimental effects had been found for exposure to the common-sole waveform used in pulse trawling, we opted for a worst-case-scenario exposure to increase the probability of detecting any potential effect (**Chapter 3**). We exposed the animals to a homogeneous electric field with high field strength (200 V m^{-1}) and a relatively long exposure duration (3 s). We found acute effects in some species such as retractions of body parts, in agreement with (Smaal and Brummelhuis, 2005; van Marlen *et al.*, 2009a; Soetaert *et al.*, 2015b, 2016d), but animals resumed normal behaviour within 30 s after exposure. We either found no effects in the quantitative locomotion behaviour assessment or found behaviour that was indicative of increased shelter behaviour. In both cases, we conclude that observed responses would not compromise survival in the wild. Furthermore, we found no effect of stimulation on survival after 14 days. As the electric field penetrates the sediment (**Chapter 4**), burrowed invertebrates may be affected. Preliminary data of burrowed lugworm *Arenicola marina* (Annelida: Polychaeta) indicates that exposure temporarily decreased bioirrigation activity (5–10 min) whilst ocean quahog *Arctica islandica* (Mollusca: Bivalvia) closed or opened their valves. Both species showed no mortality (ICES, 2020a; Tiano, 2020). Marine electrofishing studies on the brown-shrimp and razor-clam waveforms found no detrimental effects of the electric field in a range of species (Polet *et al.*, 2005; Soetaert *et al.*, 2015b, 2016d; Murray *et al.*, 2016).

The North Sea houses hundreds of benthic invertebrate species. In **Chapter 3**, we focused on six species from four different phyla to assess different neuromuscular and musculoskeletal systems to extrapolate our findings to unstudied species. Although we found no differences in effects of exposure on survival, we did find differences in acute responses and locomotion behaviour between species. Common starfish (Echinodermata: Asteroidea) and serpent star (Echinodermata: Ophiuroidae) seemed oblivious to the stimulus whilst common whelk (Mollusca: Gastropoda), sea mouse (Annelida: Polychaeta), and common hermit crab and flying crab (Arthropoda: Malacostraca) did show responses. Our findings, and those of other studies, imply that the diversity of body plans in benthic invertebrates results in susceptibility differences. The electrical stimulus is therefore likely to have a different effect on underlying physiological mechanisms in these groups. For example, Echinodermata, which have a hydrostatic water-vascular system, radial nerve net, and muscle-controlled tube feet for locomotion did not respond to the electric field (Moore, 1910; Smith, 1937; Nichols, 1972). In contrast, the other groups, which have ganglia and peripheral nerves, a muscular foot (Gastopoda; Bivalvia), or circular and longitudinal muscles to elongate and shorten body segments (Annelida), or extensor and flexor muscles in body segments (Crustacea) for locomotion (Cattaert and Edwards, 2017; Hartenstein, 2017; Kristan, 2017), did respond to the electric field. Effect of electrical stimulation on species groups with other body plans, however, have yet to be studied.

7.1.3 Marine fishes

To quantify the effect of electric fields on marine fishes, we divide the fish responses into zones with respect to the electrode arrays, similarly as done in freshwater electrofishing research (section 7.1.1; **Chapter 2**; (Snyder, 2003; Polet, 2010). From a static perspective, this categorisation of zones is also applicable to electrotrawls, and thus enables us to delineate a safety zone around the fishing gear and, closer to the electrode arrays, define distances that would induce a behavioural response, muscle activity, and internal injuries. Knowledge on threshold levels for the different responses also allows us to quantify the trawl width over which the pulsed electric field may affect marine fishes. In this section, we discuss each of these responses in relation to electric fields fishing gear and address other concerns of effects on fish that could not be incorporated in the categorisation of response zones.

In **Chapter 4**, we quantified behavioural response thresholds in five fish species at 6–10 V m^{-1} with no difference between electroreceptive and non-electroreceptive fishes. These thresholds relate to a distance of maximally 80 cm from the electrode arrays which suggests that electric fields in pulse trawling for common sole are unlikely to elicit a behavioural response outside the trawl path. In addition, we found no evidence that electroreceptive fish should be sensitive to the pulsed electric field based on the frequency sensitivity of the ampullae of Lorenzini and frequency spectrum of the stimulus. In our analyses, we ignored the interaction of fish and water due to conductivity differences (section 7.1.1). We used field strength values in our experimental setup and around the simulated electrode arrays as if no fish was present, to translate our findings from the laboratory to the field (i.e. hereafter referred to as 'external field strengths' *contra* 'internal field strengths' when we used values inside a simulated fish). Furthermore, we determined the response for the location with the highest field strength, i.e. the body part to the closest electrode, based on evidence that the effect of the electric field is local. In reality, the body of the fish will deform the electric field. We assumed that this effect would be the same in our experimental setup as *in situ* around an electrode array, in particular because water conductivities were similar. In addition, we found significant, albeit arguably irrelevant with respect to the fishing gear, species-specific differences irrespective of body length although our intraspecies length variation was limited. Future studies should identify the sources of the observed interspecies differences in behavioural response thresholds. For now, we have no indication that certain fish species would be particularly sensitive to the electric field. In addition, because behavioural thresholds are the most conservative estimate for a maximum response distance to the electrodes, we hypothesise that all responses of, at least fishes, are restricted to the trawl path.

After behavioural responses, muscle activity thresholds define the next 'zone' when moving closer to the electrodes. Preliminary data of Atlantic salmon (*Salmo salar*) revealed that involuntary muscle contraction thresholds were between 15–30 V m^{-1} as internal field strength (ICES, 2020a). This response is similar to muscle twitches. Larger fish had a lower threshold which was in line with modelling data of fish in an electric field similar as in **Figure 7.1**, where larger specimens had higher internal field strengths (ICES, 2020a). In addition, we only observed muscle twitches in the body parts closest to the electrodes, piling to the evidence that the response of a fish is determined by local electric fields. These muscle contraction thresholds relate to a maximum distance of 40 cm to an electrode in the lateral direction for a round fish at 10 cm above the seafloor (**Figure 7.1c**). Whole-body muscle contractions in Atlantic cod occur above 37 V m^{-1} as external field strength (de Haan *et al*., 2016), which should be restricted to within 30 cm around the electrodes (**Figure 4.5**). We have no data on other species. Future studies should systematically quantify the effect of parameters on thresholds as this will aid in building a predictive framework for electrical-stimulation effects on marine organisms. Nonetheless, even if thresholds would vary between species, they will likely not be lower than the thresholds we found for a behavioural response and will therefore still be limited to the trawl path.

Whole-body muscle contractions, in turn, may lead to internal injuries by fracturing and dislocating the spinal column and rupturing blood vessels. Various laboratory and field studies have demonstrated that Atlantic cod is sensitive to these electrical-pulse-induced injuries (van Marlen *et al*., 2014; de Haan *et al*., 2016; Soetaert *et al*., 2016c, 2016b, 2016a). De Haan *et al*. (2016) predicted a spinal-fracture and haemorrhage probability of 50% at 80 V m^{-1} (95% CI: 60–110 V m^{-1}) for a specimen of 50 cm (external field strength). The thresholds zone for internal injuries would in this case be restricted to within 20 cm around the electrodes (**Figure 4.5**). However, small specimens (≤17 cm) were not injured (n = 132) suggesting an effect of fish length. The presence of pulse-induced injuries in Atlantic cod, and an injury found in one wild-caught whiting specimen by a pulse trawler (van Marlen *et al*., 2014) in contrast to absence of injuries in laboratory-exposed common sole and European seabass (Soetaert *et al*., 2016b, 2018), led to the hypothesis that Gadidae might be sensitive to muscle-cramp-induced injuries.

To assess the 'Gadoid hypothesis' in **Chapter 5**, we sampled whiting catches on board pulse trawlers with and without electrical stimulus, and conventional tickler chain trawlers and assessed internal injuries. In addition, we similarly sampled fifteen other fish species presented in **Chapter 6** (together with the whiting from **Chapter 5**), such as the gadoid bib, and species with different body shapes which we assessed for spinal injuries. We only found a clear indication of electrical-pulse-induced injuries in Atlantic cod, with a length effect where larger specimens (>40 cm) were less sensitive and an indication that smaller specimens (<20 cm) might be less sensitive. Based on our findings, we rejected the hypothesis that Gadidae in general are highly sensitive to pulse injuries as we found no evidence for bib and whiting. In sandeels, we observed high injury probabilities in the sample from pulse trawlers with electricity turned on and conventional trawlers. Laboratory exposure, however, showed that sandeels are insusceptible to pulse-induced injuries (ICES, 2020a). Although we analysed sixteen species, the North Sea is inhabited by about 200 fish species (**Chapter 1**). The collected species, however, covered a range of body shapes and sizes and are representative for the majority of fish species.

We know that internal injuries occur in Atlantic cod, but have insufficient understanding of the underlying mechanisms. Studies have, so far, not been able to provide a conclusive mechanistic explanation why these injuries occur in Atlantic cod and why incidence would be fish-length dependent. We propose some experimental approaches to unravel susceptibility of Atlantic cod to internal injuries. These may also shed light on the injury incidence as function of fish length and might enable predicting effects for species resembling Atlantic cod but that were not studied as they are uncommon the southern North Sea, including haddock (*Melanogrammus aeglefinus*), pollock (*Pollachius pollachius*), and saithe (*Pollachius virens*). Because muscle contractions induced by the electrical stimulus are certainly the cause for injuries, further study on the functional morphology of muscles, ligaments, and skeleton is necessary. These include morphometric analyses of Atlantic cod specimens with different sizes and subsequent comparison with a range of other fish species of different size and shape.

Preliminary analyses by students at the Experimental Zoology Group indicate that vertebral-column characteristics (spinal angle, number of vertebrae, vertebrae width and height, and inter-vertebra distance) and muscle-to-vertebra ratio (cross-sectional area) alone do not explain sensitivity of Atlantic cod. The muscle distribution along the anteroposterior axis appears to be worthwhile for further exploration. The specific ratio of asymmetrical epaxial and hypaxial muscle distribution in the abdominal region due to the organ cavity in contrast to the rather symmetrical post-anal muscle distribution might render Atlantic cod vulnerable to injuries at the posterior abdominal and anterior caudal location. The susceptibility of Atlantic cod is presumably the result of a combination of factors. Thus, a holistic approach is required to pinpoint the mechanism. Detailed morphometric analyses of spinal fractures from regular X-radiographs and X-ray microtomography 3D reconstruction may provide insights in the mechanism of how the vertebral column collapses in contrast to our rather course quantification of injuries along the anteroposterior axis in **Chapter 6**. Finally, to visualise the fracturing of the vertebral column in real-time, one could film Atlantic cod with regular X-rays during exposure or, more technically challenging but potentially yielding more insights, with X-Ray Reconstruction of Moving Morphology (XROMM). The latter technique has been used to, amongst others, visualise skull movements in fish during feeding (Camp and Brainerd, 2015; van Meer *et al.*, 2019), and could perhaps be applied to skeletal movements of Atlantic cod during electrical stimulation.

Apart from morphological characteristics, fish behaviour could also, at least partly, explain injuries in Atlantic cod, and might also be used to avoid injuries using fishing-gear innovations. Atlantic cod is known to dive towards the seafloor in response to an oncoming trawl (Krag *et al.*, 2010), in contrast to whiting, which rises (Holst *et al.*, 2009; Krag *et al.*, 2009a). In case of pulse trawling this indicates that Atlantic cod more likely passes through the higher electric field strengths close to the electrode arrays, and therefore likely experiences more excessive muscle contractions (see above for the whole-body muscle contraction threshold). Fundamental knowledge on the mechanism behind internal injuries in Atlantic cod could provide new insights in mitigating this effect.

Apart from the above thresholds for behaviour, muscle activity, and internal injuries, fishes may be affected by the electric field in various other ways that could not be categorised in the previous sections. In the following paragraphs, we address and discuss some of these specific concerns. First, concerns on the effect of repetitive exposure on fishes were raised even though repetitive exposure at sea is low (ICES, 2020a), unless fish would be attracted to pulse gears from afar. We neither found, however, that behavioural response thresholds would indicate electric-field detection from beyond the netting around the electrode arrays, nor did we find habituation effects in electroreceptive and non-electroreceptive fish exposed to pulsed electric fields for 20–277 times within a day (**Chapter 4**).

Second, regarding external injuries, concerns rose when wild dab were found with skin ulcerations (Devriese *et al.*, 2015). Pulse-induced skin ulcerations, however, were not found in laboratory experiments (de Haan *et al.*, 2015; Soetaert *et al.*, 2016b). Vercauteren *et al.* (2018, 2019) determined that the bacterium *Vibrio tapetis* caused the ulcerations in dab, although damaged skin appeared to affect specimen's susceptibility.

Finally, concerns exist on the exposure effect on early-life stages as well as maturing fish specimens. Although eggs and larvae of the majority of North Sea fish species are pelagic, fish such as elasmobranchs and Atlantic herring (*Clupea harengus*) deposit their eggs on the seafloor (Heessen *et al.*, 2015). Studying the effects of electrical stimulation on early-life stages of these species is challenging because either housing and spawning in captivity is difficult, or collection of a sufficiently large sample size is troublesome. As an alternative, Desender *et al.* (2017b) exposed Atlantic cod embryos, larvae, and juveniles to a worst-case-scenario brown-shrimp pulse waveform. They found effects on survival and development for two out of eight developmental stages, and conclude that delayed hatching and deceased survival of larvae might indicate an effect of the stimulus. Hereafter, Desender *et al.* (2018) found no effect in a similar exposure experiment on short-term mortality, deformations, reduced growth, and yolk-sac resorption of common-sole embryos and larvae. Natural mortality of fish larvae is, however, high as the vast majority die before reaching the juvenile stage, often due to starvation (Hjort, 1914; Houde, 2002). Furthermore, in case of common-sole pulsing, electric fields are generally not present higher in the water column and are thus unlikely affect any pelagic organisms. The electrode arrays turn on at more than 25 m of the fishing line and power cable supplying the pulse modules and takes about one minute to start (pers. comm. Harmen Klein Woolthuis of HFK Engineering B.V.). In practise, the fishing gear will be at the seafloor because the southern North Sea is relatively shallow (Heessen *et al.*, 2015). During hauling, the system turns off at less than 20 m fishing line or 20 m power cable, potentially shortly leading to electric fields in the water column, depending on water depth. These data apply to PulseWing gears, which were used by two-thirds of the common-sole-targeting pulse fleet (Rijnsdorp *et al.*, 2021). Nonetheless, if effects on pelagic species remain a concern, settings to activate and deactivate the electrode arrays should be adjusted to make sure that arrays are only active on the seafloor. Finally, the effect of pulse exposure on e.g. egg quality in maturing fish has not been studied, which is potentially also highly challenging to effectuate. We see, however, no evidence that fish recruitment in the southern North Sea has changed after 2007 as a results of the introduction of pulse trawls (ICES, 2020b).

7.1.4 Concluding remarks

Based on the preceding chapters and sections in this general discussion, we have no evidence that organisms are affected by the electric field beyond the netting material around the electrode arrays. Electric field strength thresholds for behavioural responses, muscle activity, and internal injuries in fish are all restricted to the trawl path. Although Atlantic cod is sensitive for muscle-cramp-induced injuries, no substantial negative side effects of electrical stimulation were found. By defining zones based on thresholds for different responses around the electrode arrays, one can assess the effect of electrical stimulation on organisms. This approach could function as a predictive framework. Overall, we conclude that (pulsed) electric fields are a promising stimulation technique for the capture of marine organisms.

7.2 Electrofishing by fish: inspiration from nature

Humans are not the first to apply electricity in fishing. Instead, numerous fish species utilise different electrofishing techniques. As discussed in **Chapter 4**, passive electroreceptive fishes detect bioelectric fields emanated by prey hidden in the sediment (Bedore and Kajiura, 2013). In contrast, active electroreceptive fish species identify deformations in the electric field generated by themselves (i.e. they are electrogenic) (von der Emde, 1999). Finally, electrogenic fishes may also produce high-power electric strikes to e.g. capture prey (Catania, 2019). Electrofishing by fish can be subdivided into various principles that could be applied in development of electrofishing technologies for humans.

As Job Baster in 1765 questioned the effect of electricity on shrimp, he pointed out that electric eels (*Electrophorus* spp.) are able to generate electric shocks (**Chapter 1**). This early record to use inspiration from nature for solving a human problem (in Baster's case, the capture of shrimp) is what we would now refer to as 'biomimetics'. Biomimetics is the development of novel technological applications by systemically studying principles of biological systems (Lepora *et al.*, 2013). As generally well-adapted structures and mechanisms have evolved in organisms through natural selection, the field of biomimetics utilises these biological systems to acquire knowledge for technical solutions. Electrofishing mechanisms in fish have evolved multiple times independently in different groups, in both freshwater and marine species (Alves-Gomes, 2001; Crampton, 2019). These convergent systems make them an especially interesting study group as these allow for comparisons.

Section 7.1 and **Chapter 1** highlight the need for fishing techniques with minimal environmental effects. We may turn towards electroreceptive and electrogenic fishes for inspiration to optimise (electro)fishing. Passive electroreceptive fish typically detect electric fields with a low frequency and amplitude (Peters *et al.*, 2007; Collin, 2010). In contrast, electroreceptors of weakly electrogenic fish are tuned to the higher frequencies of their autogenous electric organ discharge and those of conspecifics (Crampton, 2019). In both cases, animals can detect prey (Kalmijn, 1982; Nelson and Maciver, 1999). By engineering sensors based on these two types of electroreceptors, it may be possible to detect e.g. buried flatfish. Finally, strongly electrogenic fish may be studied for their electric organ discharge waveform characteristics and hunting behaviour. For example, electric eels activate motor neurons of their prey to immobilise them but can also emit short discharge volleys that force hidden prey to reveal their location (Catania, 2014). Furthermore, electric eels concentrate their electric field through specific positioning of their body, by pushing the prey between the head and tail of the eel (Catania, 2015). The Pacific electric ray (*Torpedo californica*), likewise uses a specific body posture, named pectoral-fin cupping, in combination with a powerful electric organ discharge to, presumably, concentrate the electric field near the prey (Lowe *et al.*, 1994). The potential of studying electrofishing fish for human applications is three-fold: (i) to gain ideas and means for the detection of invertebrates and fishes (which can also work in turbid water); (ii) to improve electrical waveforms for the stimulation of organisms; (iii) to increase our fundamental knowledge on how these fishes function which may lead to unexpected discoveries and, consequently, may result in new innovations we cannot yet think of. Accurate detection and subsequent targeted stimulation of organisms may aid us in improving fishing gear selectivity.

7.3 Marine electrotrawling, quo vadimus?

As shown in this thesis, studying the effects of electrical stimulation on aquatic organisms and electrofishing is an exciting research field which involves a multidisciplinary approach with fundamental and applied science aspects. Fortunately for researchers, multiple holy grails remain to be elucidated. Although our knowledge of electrical stimulation effects on marine animals is growing and negative effects of electrotrawling compared to conventional catch methods are limited, a comprehensive, fundamental understanding of electric fields on organisms is lacking. In addition, fishing-gear developments in terms of detection of organisms, targeted stimulation, and design of electrode arrays as well as electrical waveforms are promising to improve selectivity and potentially mitigate internal injuries in Atlantic cod. In the following sections, we highlight perspectives to address some aforementioned knowledge gaps and refine pulse gears with technical innovations. These approaches may increase our knowledge on the effects of electrical stimulation on marine organisms.

7.3.1 Application of numerical simulations

Numerical modelling provides a range of opportunities in the development of electrotrawls and study of animal responses to electric fields. In this thesis, we used numerical simulations to quantify the electric field in our experimental setup and around a commercial electrode-array pair (**Chapter 4**). Technical advances of the past decades, especially in terms of computing power, have unlocked possibilities to study the deformation of the electric field by, e.g., organisms (**Figure 7.1**). Although *in situ* electric field measurements are relevant to check and quantify the electric field in experimental setups (**Chapter 4**; Polet *et al.*, 2005; de Haan *et al.*, 2016; Murray *et al.*, 2016), computer simulations can be applied more versatilely. Drawbacks of in situ field measurements include: (i) labour-intensive work when using single measurement probes that need to be moved; (ii) construction of frames with many sensors to measure multiple positions at once but with the frames potentially deforming the field; (iii) issues related to utilising electrical components in a highly corrosive environment. Furthermore, *in situ* electric field measurements are restricted in their spatial resolution as the electric field is measured between the measurement probe electrodes (**Supplementary material 4.2**) while more accurate point values can be calculated in a simulation.

Quantification of electric field strengths in fish with measurement probes is highly challenging, if not, highly annoying, due to e.g. the presence of bones and a swim bladder, and is therefore hard to standardise. Finally, measuring the *in situ* electric field is not always insightful as voltage gradients are independent of water (or sediment) conductivity (**Figure 4.3**; Reynolds and Kolz, 2012). For determining the effect of the electric field on the organism, however, one should take the conductivity of the environment and organism into account. As shown in section 7.1.1, numerical simulations can take interactions into account of an organism in the sediment and water column. Furthermore, these simulations offer the possibility to easily study the effect of body shape and size as well as orientation in an electric field (ICES, 2020a). For example, a prevailing thought is that a perpendicular orientation within a heterogenous field of an electrode pair triggers the largest response due to a maximum voltage difference over the fish's body. Although the observation is correct, the underlying mechanism explaining the response is presumably not the head-to-tail potential difference but simply a result of having body parts at higher field strengths, closer to the electrodes.

A main challenge when simulating organisms in an electric field, is to determine the correct conductivities of their components. For fish in freshwater electrofishing studies involving PTT, people have used conductivity values of 46–204 μS cm^{-1}, uniform for the body (Kolz and Reynolds, 1989; Miranda and Dolan, 2003; Kolz, 2006; Bearlin *et al.*, 2008), but skin presumably has different conductivities depending on e.g. scale type and may act as a capacitor (Beaumont, 2016). Data from these simulations (with or without fish) can be subsequently combined with thresholds for e.g. behavioural response thresholds (**Chapter 4**) and muscle activity thresholds (ICES, 2020a). Potentially, combined with laboratory experiments, the effect of pulse parameters could be studied on these thresholds. An advantage of this method is the reduction of laboratory animals as one can first test predictions with simulations. Finally, future studies could utilise numerical modelling to quantify the dynamic waveform pattern an organism experiences when passing the electrode arrays which may lead to insights on how to minimise exposure when developing gear modifications.

Above numerical-modelling perspectives are not exclusively applicable to marine electrotrawling, but may also be relevant for freshwater electrofishing research. To our knowledge, numerical simulations have not been used in freshwater electrofishing studies. In addition, this approach may help in elucidating the underlying action mechanisms that govern responses of animals in an electric field. As such, numerical modelling could be valuable in testing PTT by complementing laboratory studies to determine internal electric fields in organisms. Finally, numerical simulations could also be used to study electrogenic fish, e.g. by modelling their emanated electric fields (section 7.2), as shown in 2D by Babineau *et al.* (2007) and Ammari *et al.* (2017).

7.3.2 Fishing gear innovation

Fishing gear innovation may help to limit negative environmental effects of bottom trawling. Technical modifications and developments offer promising opportunities to improve fishing gear selectivity and reduce impact on organisms. We propose a number of new, innovative methods to detect fish and provide a targeted stimulus plus explore the potential to reduce injuries in Atlantic cod and decrease discards.

For minimal environmental impact and sustainable harvest, targeted and selective fishing is key. Here, we paint two fishing gear innovation options to improve detection of flatfish in the sediment and, if fish are detected, provide an effective, directed stimulus. For detection, we suggest to use low voltage (<1 V) electric fields to detect (buried) fish similar as weakly electric fish use their generated electric field for electrolocation (section 7.2). In addition, by using information on the deforming electric field, we might be able to e.g. detect the size of the animal. These low-amplitude, high-frequency fields are almost certainly outside of the detection range of marine animals (section 7.1; **Chapter 4**). Subsequently, we should use a locally generated electric field that is only activated upon detection of a marketable flatfish. This way, we limit the exposure of other organisms inhabiting the seafloor. As effective stimulus, we recommend to systematically study the bending of flatfish as function of electrical stimuli via e.g. tracking software to quantify curvature for each electrical setting and fish size combination.

To reduce or even prevent injuries in Atlantic cod, we propose two gear-modification approaches. First, we suggest to divert these fish away from the electrode arrays as to avoid exposing them to the zone where field strengths exceed the spinal injury threshold. This may be challenging as this species typically enters trawls close to the seafloor (Main and Sangster, 1981; Ferro *et al.*, 2007; Krag *et al.*, 2009b, 2010; Herrmann *et al.*, 2015). Thus a technical solution should aim to repel specimens from entering the trawl with e.g. light or sound stimuli, or force the fish sidewards (e.g. a 'cod shovel'), or upwards allowing them to escape the trawl through large meshes, or pass the electrode arrays at safe distance. One could possibly also lower the trawl mouth opening or place a sieve-like barrier, which may, as side effect, result in loss of marketable catches for the fishers and give problems on rough fishing grounds. Second, we propose to modify the design of the electrode arrays. Currently, the electrodes emanate the electric field equally in all directions (**Chapter 4**). A symmetric field exposes fish in the water column to high internal electric field strengths to enable stimulation of flatfish from within the sediment (**Figure 7.1**). Instead, an asymmetric electric field that is directed into the sediment only, would reduce the high field strengths in the water column. This could be achieved by insulating the current electrodes on the top or designing new electrode elements that are insulated on top and shaped such that the fields are effectively projected into the sediment. Perhaps, the disk shape of electric rays could be used as electrode-shape inspiration (section 7.2). Different designs can be easily tested with numerical simulation techniques (section 7.3.1), before manufacturing and testing in the field. Both approaches could be combined for optimal results.

Next to modification of electrode arrays in front of the trawl, placing electrodes inside the trawl is worthwhile investigating when implemented with benthos release panels. The idea posed by Soetaert *et al.* (2016b) involves the use of electrified benthos release panels to retain common sole but release e.g. benthic invertebrates and debris from the net. If marketable common sole is retained and bycatch released via the panel, this would improve catch quality, ameliorate on-deck sorting by reducing processing time, and decrease discards through increased selectivity. The latter reason might, considering the Landing Obligation (European Parliament and Council, 2013; European Commission, 2018, 2019), in particular be an incentive to further explore the feasibility of this fishing-gear modification.

Finally, but most importantly, collaboration with fishers is key in fishing gear innovation and when developing modifications for existing fishing techniques (Haasnoot *et al.*, 2016; Thompson *et al.*, 2019; Steins *et al.*, 2020; van Hoof *et al.*, 2020). As fishers are the end users that need to adopt the technique to provide in their living, involving them in the innovation process is essential to translate findings from the laboratory to the field. Moreover, fishers are a source of valuable knowledge and have hands-on experience by working at sea (Johannes *et al.*, 2000; Stephenson *et al.*, 2016). Via collaboration, fishers and researchers should gain new insights on the possibilities and limitations of fishing-gear innovations to improve selectivity and mitigate negative effects on the ecosystem.

7.4 Epilogue

We started this thesis with the global capture fisheries quest for sustainable harvest with minimal environmental impact. We investigated the effects of electrical stimulation on marine organisms that encounter pulse-trawl gears. First, we concisely described the marine electrofishing field and provided an approach to improve communication standards (**Chapter 2**). We gained understanding on locomotion behaviour responses and survival probabilities of marine benthic invertebrates exposed to electrical pulses (**Chapter 3**). Furthermore, by quantifying behavioural response thresholds of electroreceptive and non-electoreceptive fishes (**Chapter 4**), and by quantifying internal injuries in sixteen fish species (**Chapter 5** and **6**), we conclude that the effects of the electrical stimulus are negligible compared to the mechanical disturbance of trawl gears for targeting common sole. Although Atlantic cod is sensitive to electrical-pulse-induced injuries, we see opportunities to mitigate this negative side-effect.

Apart from direct effects of the electrical stimulus on marine organisms – the main focus of this thesis – one should also consider other direct, ecological, and environmental effects of pulse trawling and compare these with other fishing methods. Some of these effects have been addressed in the Impact Assessment Pulse-trawl Fishery (IAPF) project (**Chapter 1**) (Rijnsdorp *et al.*, 2020a; Tiano, 2020), whilst, for example, some other effects have been studied in the Benthic Ecosystem Fisheries Impact Studies (BENTHIS) project (Rijnsdorp *et al.*, 2017). Only by taking into account the aforementioned topics, one can fully assess and compare the impact of different catch methods on the ecosystem and environment.

Catching marine organisms with electric current in the European Union is prohibited and temporary derogations for pulse trawls have been revoked per 30 June 2021 (Council of the European Union, 1998; European Parliament and Council, 2019), but scientific research with vessels is permitted under certain conditions. Based on the research presented in this thesis, we see potential to improve and refine pulse trawls and, therefore, think it would be worthwhile to further investigate such capture techniques. In general, we think it is important to create a broad support for development and improvement of fishing-gear techniques, because sustainable development should take environmental, economic, and social aspects into account. Hereto, we propose to make fishing-gear-innovation guidelines for weighing the advantages and disadvantages in balancing the environmental, economic, and social impacts with sustainable harvest. The findings presented in this thesis add to a scientific basis to compare and evaluate pulse-trawl impact on the marine ecosystem with other fishing techniques.

7.5 References

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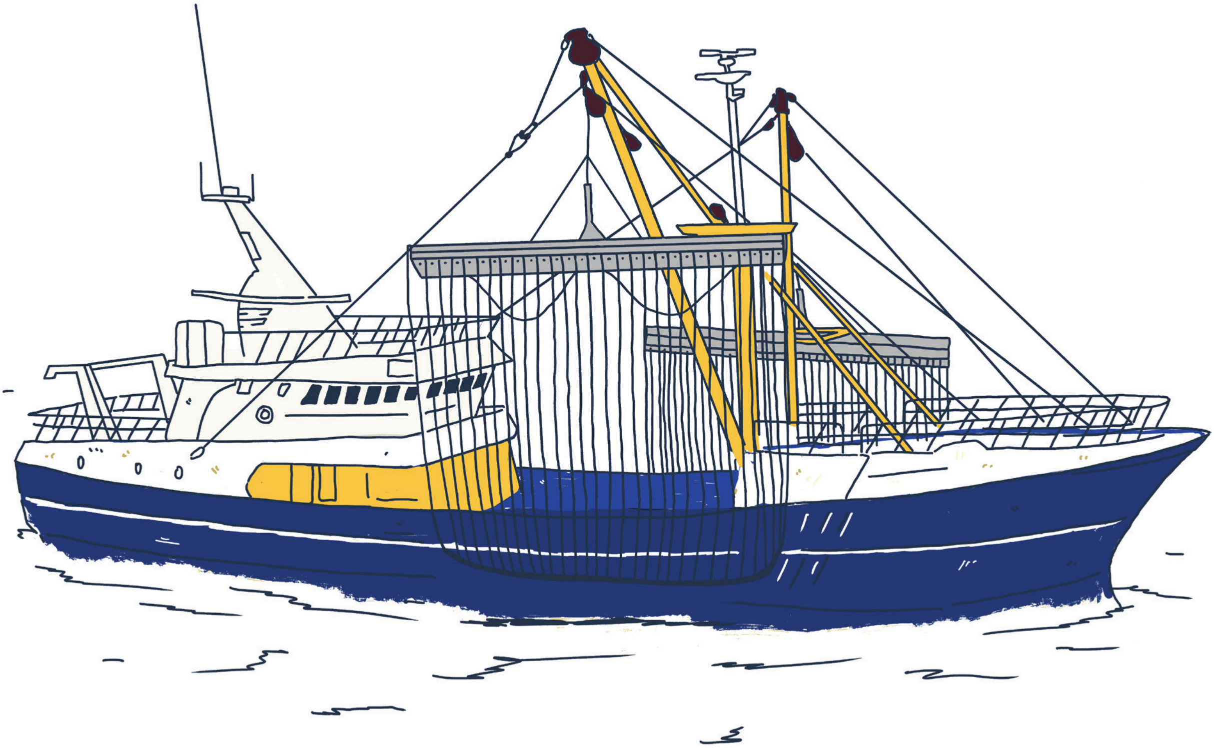


Summary
Samenvatting

Summary

Marine capture fisheries are important in providing food and livelihoods globally. A common fishing method is bottom trawling, which involves dragging nets over the seafloor to capture benthic invertebrates and fishes. In Northwestern Europe, beam trawls with tickler chains are used to catch the flatfish species common sole (*Solea solea*) in the North Sea. This technique, however, is characterised by poor selectivity, large disturbance of the benthic ecosystem, and high fuel consumption. An alternative catch method is to replace the tickler chains by electrode arrays which generate pulsed electric fields. This electrical stimulus induces involuntary muscle contractions which immobilise fishes and enables subsequent capture. Pulse trawling raised concerns about potential negative impacts on marine organisms. In this thesis, we examined effects of electrical pulse stimulation on benthic invertebrates and fishes and explored the ecological implications of electrotrawling.

In **Chapter 2**, we reviewed the marine electrotrawling field, described physiological responses of organisms exposed to electrical stimulation, and outlined electrical waveform characteristics. Based on published literature, we identified a lack of consistency in the description of electrical parameters in marine electrofishing laboratory and field research. Here, we offered recommendations for better communication standards in electrofishing and pulse trawling in particular. Specifically, we aimed to standardise descriptions of electrical waveform parameters, experimental designs, and environmental parameters. Our work may also promote collaboration with the freshwater electrofishing research community.

In **Chapter 3**, we studied effects of electrical pulse exposure on benthic invertebrates. In particular, we quantified changes in locomotion behaviour that might increase predation risk. We also scored acute behaviour during exposure and subsequent recovery period to reveal potentially different response mechanisms between species. Furthermore, we monitored survival up to 14 days after exposure. We examined these responses in six species from four phyla, namely common starfish (*Asterias rubens*), serpent star (*Ophiura ophiura*), common whelk (*Buccinum undatum*), sea mouse (*Aphrodita aculeata*), common hermit crab (*Pagurus bernhardus*), and flying crab (*Liocarcinus holsatus*). Responses during stimulation varied from no visible effect (echinoderms) to squirming (sea mouse) and retractions (whelk and crustaceans). All animals resumed normal behavioural patterns, without signs of lasting immobilisation within 30 s after stimulation. We found no change in locomotion patterns after stimulation for starfish, serpent star, whelk, and sea mouse. In contrast, flying crab and hermit crab showed significant changes in activity that were indicative of increased shelter behaviour. We found no effect of electrical

exposure on survival after 14 days in all species. These findings suggest that changes in locomotion behaviour due to electrical stimulation as used in pulse trawling are unlikely to substantially compromise survival of the investigated species.

In **Chapter 4**, we addressed concerns that the electric fields of pulse trawls may affect fishes outside the trawl track. We measured behavioural response thresholds for electric field strengths in the laboratory and compared these thresholds to computer-simulated field strengths around electrode arrays of a commercial pulse trawl. We assessed thresholds for electroreceptive small-spotted catshark (*Scyliorhinus canicula*) and thornback ray (*Raja clavata*) as well as non-electroreceptive European seabass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*), and common sole. Thresholds for different species varied between 6.0 and 9.8 V m^{-1}, with no significant difference between electroreceptive and non-electroreceptive species. These thresholds correspond to a distance of maximally 80 cm from the electrode arrays of the simulated electric fields around the fishing gear. Our findings suggest that electrical pulses as used in pulse trawling are unlikely to elicit behavioural responses outside the nets that surround the electrode arrays.

In **Chapter 5**, we examined the hypothesised susceptibility of Gadidae for pulse-induced injuries by quantifying internal injuries in whiting (*Merlangius merlangus*) catches. We sampled specimens from pulse trawls with and without electrical stimulation, and conventional beam trawls with tickler chains to shed light on the injury origin. We visualised spinal injuries with X-radiography, followed by dissection to reveal internal haemorrhages. Both injury types were categorised on a severity scale and their location was quantified along the anteroposterior fish axis. Spinal injury probabilities in pulses-on and pulses-off catches were low (on average ≤3%) and we found no evidence for electrically-induced injuries. Severe spinal injury probability was slightly higher in tickler-chain catches (2.5%) than in pulses-on samples (0.8%) and this difference increased for smaller specimens. The locations of spinal injuries did not show a consistent pattern as previously shown in Atlantic cod exposed to electrical pulses in laboratory conditions. Severe haemorrhage probabilities were also low, but slightly higher in the pulses-on samples (1.8%) compared to fish caught with tickler chains (0.3%), especially for the larger specimens. The locations of severe haemorrhages in pulses-on catches, and a correlation with spinal injury occurrences, suggest that they may be (partly) related to electrical-pulse exposure. Overall, our results indicate that spinal injuries in whiting are rare and primarily due to mechanical impact. Severe haemorrhages may be partially related to electrical pulsing but incidences are low and coincide with a significantly lower chance for spinal injuries. Based on these findings, we rejected the hypothesised susceptibility of Gadidae for pulse injuries in general.

In **Chapter 6**, we focused on concerns about potential spinal injuries in fish species caught with electrical pulses. To quantify spinal injuries, we examined sixteen, widely different, fish species from catches of tickler-chain trawlers and electrical-pulse trawlers. Sampled species included common sole, dab (*Limanda limanda*), European plaice (*Pleuronectes platessa*), solenette (*Buglossidium luteum*), Atlantic cod, bib (*Trisopterus luscus*), whiting, grey gurnard (*Eutrigla gurnardus*), tub gurnard (*Chelidonichthys lucerna*), lesser sandeel (*Ammodytes tobianus*), greater sandeel (*Hyperoplus lanceolatus*), bullrout (*Myoxocephalus scorpius*), dragonet (*Callionymus lyra*), European seabass, lesser weever (*Echiichthys vipera*), and striped red mullet (*Mullus surmuletus*). To distinguish mechanically and electrically-induced injuries, we compared, for a subset of species, injuries in samples from pulse gears with electrical pulses either turned on or off. Severity of spinal injuries and their location along the anteroposterior fish axis were quantified from X-radiographs. Except for Atlantic cod and sandeels, spinal injury probability was low (<2.5%), irrespective of severity category and catch method. In sandeels, we found no evidence for electrically-induced injuries. In Atlantic cod, 40% had major spinal injuries in pulses-on samples versus 1% in tickler-chain samples. Both the location of injuries in the pulses-on samples and fish-length dependency of injury incidences, match findings for Atlantic cod in laboratory experiments. Overall, our results show that electrically-induced spinal injuries as present in Atlantic cod are not found in a wide range of other bycatch species of common-sole-targeting bottom trawling. Apart from Atlantic cod, pulse trawling is therefore unlikely to impose increased mortality on studied fish populations compared to the tickler-chain technique.

Finally, in **Chapter 7**, we placed our findings in a wider scientific context. We integrated the most important thesis outcomes with the existing knowledge regarding effects of electrical stimulation on marine animals. To assess the effect of electrical stimulation on organisms in a mechanistic framework, we defined zones based on thresholds for different responses around the electrode arrays. We found no evidence that organisms are affected by the electric field beyond the netting material around the electrode arrays. Electric field strength thresholds for behavioural responses, muscle activity, and internal injuries in fish are all restricted to the trawl path of the gear. No substantial negative side effects of electrical stimulation were found. Hereafter, we explored the biomimetic potential of electroreceptive and electrogenic fish species. In particular, we provided an outlook on the design of novel electrical detection and stimulation possibilities for fishing. We presented future research perspectives with numerical simulation and fishing gear innovation. Although Atlantic cod is sensitive to electrical-pulse-induced injuries, we suggest ways to mitigate this negative side-effect through gear modifications. In conclusion, we see potential to improve and refine pulse trawls and, therefore, think it would be worthwhile to further investigate such capture techniques.

Samenvatting

Zeevisserij is belangrijk in de globale voedselvoorziening en het levensonderhoud van mensen. Een veelvoorkomende visserijmethode is de zogenaamde demersale sleepnetvisserij (ook wel korren genoemd). Bij deze vismethode worden netten over de zeebodem gesleept om vissen en benthische ongewervelden te vangen. In Noordwest Europa worden zogenaamde boomkorren met wekkerkettingen en kietelaars (hierna: kettingen) gebruikt om de platvissoort tong (*Solea solea*) te vangen in de Noordzee. Deze techniek wordt echter gekarakteriseerd door een geringe vangstselectiviteit, een aanzienlijke verstoring van het benthische ecosysteem en een hoog brandstof verbruik. Een alternatieve vangstmethode is het vervangen van de kettingen door elektrodenstrengen die gepulste elektrische velden genereren. Deze elektrische stimulus induceert onvrijwillige spiersamentrekkingen waardoor vissen geïmmobiliseerd worden en vervolgens gevangen kunnen worden. Deze zogenaamde pulskorvisserij heeft echter tot zorgen geleid omtrent potentiële negatieve invloeden van de elektrische stimulering op mariene organismen. In dit proefschrift onderzochten we de effecten van elektrische puls stimulatie op benthische ongewervelden en vissen en verkenden we de ecologische implicaties van deze elektrosleepnetvisserij.

In **Hoofdstuk 2**, presenteerden we een overzicht van het elektrische sleepnetvisserij onderzoeksveld, beschreven we de fysiologisch responsies van organismen die blootgesteld worden aan elektrische stimulatie en hebben we de elektrische golfvormkarakteristieken uiteengezet. We identificeerden, gebaseerd op gepubliceerde wetenschappelijke literatuur, een gebrek aan consistentie in de beschrijvingen van elektrische parameters in het laboratorium- en veldonderzoek omtrent mariene elektrovisserij. Hier boden we aanbevelingen aan voor verbeterde communicatie standaarden in elektrovisserij en pulskorvisserij in het bijzonder. We streefden specifiek naar het standaardiseren van beschrijvingen van elektrische golfvorm parameters, experimentele ontwerpen en omgevingsparameters. Ons werk zou ook de samenwerking met de zoetwater elektrovisserij onderzoeksgemeenschap kunnen bevorderen.

In **Hoofdstuk 3** bestudeerden we de effecten van elektrische puls blootstelling op benthische ongewervelden. In het bijzonder hebben we veranderingen in voortbewegingsgedrag gekwantificeerd die mogelijk tot een verhoogd predatatierisico kunnen leiden. We hebben tevens acute gedragsveranderingen gescoord tijdens de elektrische blootstelling en gedurende de daaropvolgende herstelperiode om mogelijk verschillende responsiemechanismen tussen soorten te duiden. Daarnaast hebben we de overleving van de dieren tot en met 14 dagen na blootstelling gemeten. We onderzochten deze responsies in zes diersoorten komende uit vier fyla, namelijk de gewone zeester (*Aste-*

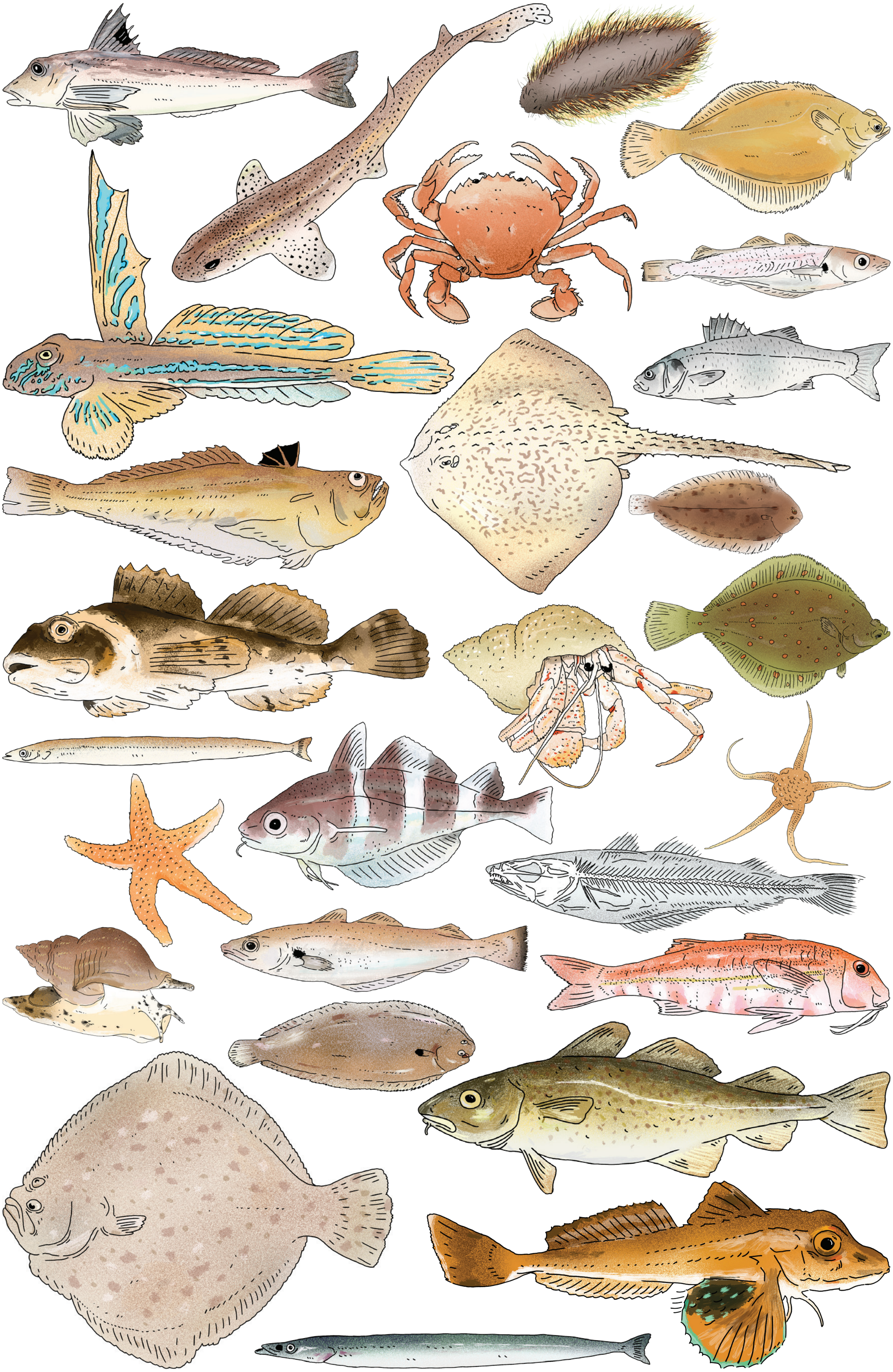
rias rubens), gewone slangster (*Ophiura ophiura*), wulk (*Buccinum undatum*), fluwelen zeemuis (*Aphrodita aculeata*), gewone heremietkreeft (*Pagurus bernhardus*) en gewone zwemkrab (*Liocarcinus holsatus*). Responsies tijdens de stimulatie varieerden van geen zichtbaar effect (stekelhuidigen) tot kronkelen (fluwelen zeemuis) en intrekkingen (wulk en kreeftachtigen). Alle dieren hervatten normale gedragspatronen binnen 30 seconden na blootstelling, zonder tekenen van blijvende immobilisatie. We vonden geen verandering in voortbewegingspatronen na stimulatie in de gewone zeester, gewone slangster, wulk en fluwelen zeemuis. Bij de gewone heremietkreeft en gewone zwemkrab vonden we significante veranderingen in activiteit die indicatief waren voor verhoogd schuilgedrag. In geen van de soorten vonden we een effect van de elektrische blootstelling op de overleving na 14 dagen. Deze bevindingen suggereren dat het onwaarschijnlijk is dat veranderingen in voortbewegingsgedrag als gevolg van elektrische stimulatie zoals gebruikt door de pulskorvisserij de overleving van de onderzochte soorten in gevaar zullen brengen.

In **Hoofdstuk 4** hebben we de bezorgdheid behandeld dat elektrische velden van pulskorren vissen buiten het sleepspoor zouden kunnen beïnvloeden. Hiertoe hebben we gedragsresponsiedrempels voor elektrische veldsterkten gemeten in het laboratorium en deze drempels vergeleken met computer gesimuleerde elektrische velden rondom elektrodenstrengen van een commerciële pulskor. We hebben responsiedrempels vastgesteld voor de elektroreceptieve hondshaai (*Scyliorhinus canicula*) en stekelrog (*Raja clavata*) evenals de niet-elektroreceptieve Europese zeebaars (*Dicentrarchus labrax*), tarbot (*Scophthalmus maximus*) en tong. Drempelwaarden voor de verschillende diersoorten varieerden tussen 6,0 en 9,8 V m^{-1}, zonder een significant verschil tussen elektroreceptieve en niet-elektroreceptieve soorten. Deze drempels corresponderen met een afstand van maximaal 80 cm van de elektrodenstrengen met de gesimuleerde elektrische velden rond het vistuig. Onze bevindingen suggereren dat het onwaarschijnlijk is dat elektrische pulsen, zoals gebruikt in de pulskorvisserij, gedragsresponsies veroorzaken buiten de netten die de elektrodenstrengen omgeven.

In **Hoofdstuk 5** onderzochten we de veronderstelde gevoeligheid van Gadidae voor puls geïnduceerde verwondingen, door interne verwondingen in wijting (*Merlangius merlangus*) vangsten te kwantificeren. We hebben monsters genomen uit de vangst van pulskotters die met en zonder elektrische stimulus visten evenals van conventionele boomkorkotters met kettingen om licht te werpen op de oorsprong van de verwondingen. We visualiseerden verwondingen aan de wervelkolom met röntgenopnames, gevolgd door dissectie om interne bloedingen te onthullen. Beide verwondingstypen werden gecategoriseerd op een ernstschaal en hun locatie werd gekwantificeerd op de lichaamsas van de vis. De kans op ruggengraatletsel bij puls-aan en puls-uit vangsten was laag (gemiddeld ≤3%) en we vonden geen bewijs voor puls geïnduceerde verwondingen. De kans op ernstige verwondingen aan de wervelkolom was iets hoger in de kettingvangsten (2,5%) dan in de puls-aan monsters (0,8%) en dit verschil nam toe voor kleinere exemplaren. De locatie van wervelkolomverwondingen toonde geen consistent patroon zoals eerder aangetoond bij kabeljauwen die waren blootgesteld aan elektrische pulsen in laboratoriumomstandigheden. De kans op ernstige bloedingen was ook laag, maar iets hoger in de puls-aan monsters (1,8%) in vergelijking met vissen gevangen met kettingen (0,3%), vooral voor de grotere exemplaren. De locaties van ernstige bloedingen in puls-aan vangsten en een correlatie met het voorkomen van verwondingen aan de wervelkolom, suggereren dat ze mogelijk (deels) gerelateerd zijn aan de elektrische puls blootstelling. Globaal genomen tonen onze resultaten aan dat verwondingen aan de wervelkolom in wijting zeldzaam zijn en voornamelijk komen door mechanische impact. Het aantal vissen met ernstige bloedingen die deels gerelateerd kunnen zijn aan de elektrische blootstelling is laag en gaat samen met een significant lagere kans op wervelkolomverwondingen. Gebaseerd op deze bevindingen verwierpen wij de hypothese dat Gadidae in het algemeen gevoelig zouden zijn voor puls verwondingen.

In **Hoofdstuk 6** hebben we ons gericht op de zorgen over mogelijke wervelkolomverwondingen bij vissoorten die met elektrische pulsen zijn gevangen. Om ruggengraatletsel te kwantificeren, onderzochten we zestien, zeer uiteenlopende, vissoorten uit vangsten van pulskorkotters en van boomkorkotters die met kettingen visten. Bemonsterde soorten waren tong, schar (*Limanda limanda*), schol (*Pleuronectes platessa*), dwergtong (*Buglossidium luteum*), kabeljauw, steenbolk (*Trisopterus luscus*), wijting, grauwe poon (*Eutrigla gurnardus*), rode poon (*Chelidonichthys lucerna*), zandspiering (*Ammodytes tobianus*), smelt (*Hyperoplus lanceolatus*), gewone zeedonderpad (*Myoxocephalus scorpius*), gewone pitvis (*Callionymus lyra*), Europese zeebaars, kleine pieterman (*Echiichthys vipera*) en rode mul (*Mullus surmuletus*). Om mechanisch geïnduceerde verwondingen van elektrisch geïnduceerde verwondingen te onderscheiden vergeleken we, voor een subgroep aan soorten, verwondingen in monsters van pulstuigen met de elektrische pulsen ofwel aangeschakeld dan wel uitgeschakeld. De ernst van de wervelkolomverwondingen en hun respectievelijk locatie op de lichaamsas van de vis werden gekwantificeerd op basis van röntgenopnames. Behalve voor kabeljauw, zandspiering en smelt, was de kans op verwondingen aan de wervelkolom laag (<2,5%), ongeacht de ernstcategorie en vangstmethode. Bij zandspiering en smelt vonden we geen bewijs voor elektrische puls geïnduceerde verwondingen. Bij kabeljauw had 40% van de onderzochte dieren majeure verwondingen in de puls-aan monsters in vergelijking met 1% in de kettingmonsters. Zowel de locatie van de verwondingen in de puls-aan monsters als de afhankelijkheid van verwondingen voor vislengte komen overeen met bevindingen bij kabeljauw in laboratoriumstudies. Over het algemeen laten onze resultaten zien dat de elektrische puls geïnduceerde wervelkolomverwondingen zoals aanwezig in kabeljauw, niet worden gevonden in een groot aantal andere bijvangstsoorten van de sleepnetvisserij op tong. Afgezien van kabeljauw is het daarom onwaarschijnlijk dat pulskorvisserij leidt tot een verhoogde mortaliteit van de bestudeerde vispopulaties in vergelijking met de ketting vangsttechniek.

Ten slotte plaatsten we in **Hoofdstuk 7** onze bevindingen in een bredere wetenschappelijke context. We integreerden de belangrijkste onderzoeksresultaten met de reeds bestaande kennis over de effecten van elektrische stimulatie op mariene dieren. Om het effect van elektrische stimulatie op organismen in een mechanistisch kader vast te stellen, definieerden we zones rondom de elektrodenstrengen, gebaseerd op de drempels voor verschillende responsies. We vonden geen bewijs dat dieren beïnvloed worden door het elektrisch veld buiten het netmateriaal dat zich rondom de elektrodenstrengen bevindt. Elektrische veldsterkte drempels voor gedragsresponsies, spieractiviteit en interne verwondingen in vissen worden alleen binnen de breedte van het vistuig overschreden. Er werden geen substantiële negatieve neveneffecten van de elektrische stimulatie gevonden. Hierna verkenden we de biomimetische potentie van elektroreceptieve en elektrogene vissoorten. In het bijzonder gaven we een vooruitzicht op het ontwerp van nieuwe elektrische detectie en stimulatie mogelijkheden voor de visserij. We presenteerden toekomstige onderzoeksperspectieven met numerieke simulaties en vistuiginnovatie. Hoewel kabeljauw gevoelig is voor elektrische puls geïnduceerde verwondingen, stellen we manieren voor om dit negatieve neveneffect te mitigeren door middel van vistuigmodificaties. Samenvattend zien wij potentie om de pulskortechniek te verbeteren en te verfijnen. Daarom denken wij dat het de moeite waard is om dit type vangsttechnieken verder te onderzoeken.



Acknowledgements

Acknowledgements

During my PhD project, I had the privilege and luck to meet many kind people as well as retain friendships with people I met earlier in life. Without these amazing folks, I would not have been able to finish my research and write this PhD thesis. In this section, I try to highlight the people that have meant the most to me, both professionally and personally, but know that I have insufficient words to thank all of you. Writing these acknowledgements was an amazing trip down memory lane. Although it is impossible to mention all the adventures and funny moments, I am always open to recollect these memories and to experience new activities together! It was a joy and pleasure to have met you and worked with you!

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within my project. Raoul, thank you for your dedication in helping with the sampling on board of fishing vessels, the cool stories you brought back from sea, and for your help with the fish processing, both physically and digitally. Noraly, I will never forget the funny moments during our trip to Ostend by car, loaded with ~1,500 L of seawater in tubs. Thanks a lot for your work on the physical and digital fish processing. **Hans**, electrotechnician at the Technical Development Studio, I would like to thank you for your insights and advice regarding the pulse generator equipment you constructed. I appreciate your availability for questions.

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As everybody knows, only the acknowledgements of a PhD thesis are read in detail. This section is, therefore, the excellent place to advertise the research (Voesenek, 2019; Cribellier, 2021). I highlight the experimental work done for this thesis in the following four paragraphs where I thank my students (in sequence of chapters). I hope this will encourage you, as reader, to study these chapters and learn more about the effects of electrical stimulation on marine organisms. May it spark your curiosity!

During my PhD trajectory I had the privilege to supervise many interested, motivated, and kind students. **Adrian**, you started in the summer of 2018 and worked on the effects of electrical pulsing on marine benthic invertebrates (read: creepy critters that inhabit the seafloor, which are actually beautiful and fascinating organisms). The experiments were done at the Flanders Research Institute for Agriculture, Fisheries and Food (ILVO) in Ostend, Belgium. We had a busy schedule with long days for performing our measurements. We achieved an enormous amount of work during our three months

in Ostend. I want to thank you for your perseverance and dedication! I enjoyed your passion for marine aquaria, dinners together (sole à la meunière or proper Spanish paella), and the little time we had for partying. I hope you also enjoyed your time in Ostend and Bredene! The work we did has been published and is described in Chapter 3.

Another part of this thesis focuses on behavioural response thresholds of marine fishes for pulsed electric fields. Several students have worked on this topic throughout the years. **Marleen**, you started with the fish behaviour topic in Wageningen. You helped building the setup, developed protocols, and performed pilot measurements on the behavioural responses of electroreceptive small-spotted catshark (*Scyliorhinus canicula*) and thornback ray (*Raja clavata*). I enjoyed your enthusiasm and I wish you all the best in the future! **Lara**, you joined somewhat later and continued the measurements done by Marleen and also included turbot (*Scophthalmus maximus*). I liked your motivation and drive throughout your period at EZO. Finally, **Koen** joined this subproject. Based on the pilot, we performed measurements on the three aforementioned fish species as well as common sole (*Solea solea*) and European seabass (*Dicentrarchus labrax*). We also quantified the *in situ* electric field strengths in the experimental setup. I highly appreciate your involvement with data checks after your departure. The findings are described in Chapter 4 and I aim to submit the manuscript for publication soon!

Sarina, you were my second MSc student. Your work involved processing thousands of fishes from trawler catches to assess their internal injuries. You were probably the 'smelliest' student of all. I will never forget when we were figuring out our processing protocols, that the fish smell had spread far throughout the Zodiac building. You are very energetic, pro-active, and pragmatic. From picking up fish in the port when Raoul came back from sea in the middle of the night, to joining project meetings. I enjoyed working with you! **Lisanne**, you started your BSc thesis within the fishing-fleet-sampling subproject and focused on flatfishes. You were very motivated to join our research, coming from Utrecht University, and worked very disciplined and precise. Chapter 5 and 6 contain the internal injury assessment results!

Not all work during my PhD project could make it to a research chapter. These findings, however, are partly incorporated in the general discussion of this thesis (Chapter 7). **Raoul** was my first student to join the pulse team. Raoul worked on involuntary muscle activation thresholds of Atlantic salmon (*Salmo salar*) in response to electrical stimulation. As I had started my PhD project just five months before you commenced, much was also new to me. I enjoyed going through this discovery process together. From building an experimental setup to measure muscle activation threshold in fishes, to spray painting the fleet sampling boxes behind Zodiac. After your MSc thesis, you were appointed as research assistant to help in the trawler sampling at sea and processing of fishes in the B-basement and on the computer. I sincerely enjoyed working with you! **Jasper**, you worked together with Raoul on muscle activation thresholds in fishes and completed your BSc thesis on this topic. By this time, our pulse group had grown so much that we arranged weekly meetings with Martin. I still wonder why I scheduled our meeting so early on Monday morning! It was pleasant working with you as you were always enthusiastic and creative. **Mickey** and **Amerik**, you performed an elaborate morphometric analysis on various fish species to investigate why Atlantic cod is sensitive to internal injuries as consequence of pulse-induced muscle contractions. In addition, you performed a stakeholder assessment on pulse trawling through interviews. You both worked diligently and were highly motivated. It was fun working with you! Your morphometric dataset was extended by **Clarice** and **Jesse**. I remember the fun times during our trip to Den Helder and IJmuiden to pick up fishes and I enjoyed working with you. Finally, **Joey**, you studied the effect of electrical stimulation on buried lugworm (*Arenicola marina*) in collaboration with Justin at the Royal Netherlands Institute for Sea Research (NIOZ). I enjoyed my short stays in Yerseke to work out the experimental setup together and discuss ideas for measurement procedures based on previous experience by Justin *et alii*. You are cheerful, resourceful, and diligent.

My dear student army, I hope you enjoyed your time at EZO, having me as supervisor (together with Martin, Adriaan, or Justin), and going to various pulse project and stakeholder meetings. I learned a lot from you and from supervising you. I hope your time at EZO helped you prepare for the future. I would like to thank you all for your hard work and the fun we had. I hope we will stay in touch!

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My PhD project and thesis would not have been possible without the advice, help, and support of colleagues from WMR in Yerseke, IJmuiden, and Den Helder, the Netherlands. **Pieke**, thank you for sharing your hands-on knowledge on the North Sea fisheries and fishing gear technology. I loved our trips at sea on pulse trawlers to sample fishes and work on the discard's survival study. Although we had to empty our stomach several times, the sea is calling again! Many thanks for sampling on other fishing trips and making surplus live animals available for my PhD research. **Edward**, thanks for your help with logistics and administrative businesses. I enjoyed our sandeel collaboration. Thanks for your advice and sharing your knowledge and expertise. In addition, I would like to thank all other WMR colleagues I worked with, and in particular **Dick**, **Harriet**, **Jurgen**, **Michiel**, **Nathalie**, **Pim**, and **Ruben** for your support during various stages of the research.

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My PhD project was embedded in a larger research consortium that included the NIOZ. Here, **Justin** (now dr. Tiano) was my PhD student counterpart, studying the consequences of bottom trawling on benthic-pelagic coupling and ecosystem functioning (including effects of electrical stimulation on biogeochemistry). Justin, thank you for the fun and great times during our project meetings, conference visits, and my time in Yerseke. I enjoyed our collaboration on the lugworm study involving cool technologies and supervising Joey together. **Karline**, thank you for your advice and support with our lugworm study and feedback during project meetings!

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I owe much to the members of WGELECTRA. Thanks for sharing your knowledge and insights during the past years. We discussed the ongoing marine electrofishing research during our annual meetings, published reports that were used by ICES to formulate advices, and worked out research ideas. One of these ideas became concrete when you, **Daniel**, offered me the opportunity to join the FRV Solea for pulse trawling field research starting in Cuxhaven, Germany. Thanks for taking me on board together with **Maarten**,

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When I joined EZO, I shared the office with **Julian** and **Uroš**. At first, the seemingly only common denominator of our office was that our research topics were 'miscellaneous'. Our projects focused on tree frog attachment, biomechanics of parasitic wasp oviposition, and electrical stimulation of marine organisms. It turned out, however, that we had many things in common and we quickly became friends! I enjoyed, amongst others, our styrofoam-ball fights, ACDC Fridays, and drinks. Julian, gentle giant (people are not often taller than me), I admire your discipline and thoughtfulness. Thanks for introducing me to your family (which expanded over the years) and friends in Arnhem by inviting me over for dinner, the doomsday clock party, and an amazing New Year's Eve on the John Frost bridge. **Annika**, **Sophie**, and **Johanna**, let's go to the zoo together soon! Uroš, you were a cheerful and entertaining colleague! I liked our discussions on science in the broadest sense, together with Julian, including extensive use of the white board with intelligent-looking schemes and formulas. After you finished at EZO and went to Belgium, we stayed in touch. I sincerely enjoyed visiting you in Liège. May the force be with you during your endeavours in Slovenia and I cannot wait to visit you! I wish you and **Eva** all the best! Julian and Uroš, I very much enjoyed being your paranymph. I am extremely happy that you are now my paranymphs (a.k.a. powernymphs). Although we do not work in the same office anymore, I trust we will keep in touch.

EZO is a coherent group consisting of so-called EZO-ites/EZO-ians (do we have consensus on the correct nomenclature actually?). You made me feel welcome really quickly. **Andres**, your sincerity and humbleness are staggering. I am happy you are co-author on Chapter 4. You have taught me a lot with your inexhaustible advice and support regarding statistics. It was an honour to be your paranymph. Thank you also for clubbing in Arnhem! I wish you and **Caroline** all the best, trust we stay in contact, and hope I can visit you soon in your mountain cabin in Switzerland! **Anneke**, thank you for your kindness when I was in Forum. **Antoine**, I do not know where to start and end. Infinite thanks for your friendship and all the great times we had: from walks in nature during corona, to movie nights you organised in Zodiac. **Arie**, your enthusiasm is contagious and thanks for your kindness and help when I was teaching in Forum. **Bart**, I enjoyed assisting in your course. **Camille**, it was nice to have you as visiting researcher during your PhD project and best of luck for your post-doc at EZO! **Cees** (a.k.a. Voesenek, more recently dr. Voesenek), thank you for providing me with some rhythm in life via the strict coffee break and lunch schedule that you forced upon me from day one. **Dayo**, it was nice to have you around as student and I wish you a great PhD journey at EZO! **Ellen**, thanks for the great conversations on teaching! **Elsa**, you were very kind when I met you during my master's in Groningen and I am happy to have shared the office with you in Wageningen at the beginning! **Florian**, I liked the discussions on a variety of topics with you during the breaks. **Gauthier**, thanks for the nice chats in Carus and showing me your fish. **Guillermo**, all the best with your soft robotics research! **Heleen**, thank you for the fun times when you were a student at EZO and thereafter! **Henri**, your shrimps are thriving in Groningen! **Henry**, thanks for the funny moments and all the best with making the world mosquito-free! **Kees**, thank you for the interesting stories on science and tracking software development. You have an excellent taste regarding the Chinese-Indonesian restaurant in Wageningen! **Lana**, you joined our 'miscellaneous office' a bit later. Although your topic does not entirely meet this criterium, you were a great addition to our office. I liked our chats! All the best to you and **Mark**! **Leo**, thank you for all the interesting facts you provided during the breaks. **Lisa** (fish), thanks for the fun moments! **Lisa** (frog), it was a pleasure to have such a good actress for Julian's movie. **Mike**, initially I shared the office with you, Myrthe and Elsa. Thanks for guiding me at EZO in this period and all the fun times thereafter! I hope I can visit you and **Suus** soon in your new house! **Myrthe**, thanks for your friendship and nice house parties. I look forward visiting you and **Joeri** in 020. **Noraly**, you were a great office mate, very kind and open! My best wishes to you and **Jorn** with your new house! **Pulkit**, your laugh is contagious! Thank you for all the fun activities together! **Sander K.**, thanks for the interesting perspectives you provided with your teaching experience. **Sander G.**, thanks for providing me with marine biology books! **Steffen**, although we only met shortly as you started during the pandemic, I hope you meanwhile settled within the group! **Tiffany**, your sparky energy is catchy! **Wouter van G.**, I will never forget the day when you showed me the swordfish skull you were macerating. **Wouter van V.**, I liked our coffee break discussions on nerdy topics and firing the cannon. I have fond memories of the, almost weekly, pre-climbing dinner on campus and the EZO Thank You Dinners for the support staff. I also liked our Journal Club, being impressed and inspired by work of others but simultaneously bashing papers. In addition, the writing group was fun and a great place to learn from peer-review! I am grateful for the wonderful time I had at EZO.

I want to thank the colleagues from the Cell Biology and Immunology Group and Host-Microbe Interactomics Group for making my stay in Zodiac so pleasant. **Adrià**, **Annelieke**, **Carmen**, **Edwin**, **Esther**, **Geert**, **Hilda**, **Huub**, **Joost**, **Jules**, **Julia**, **Maria**, **Marloes**, **Mark**, **Mirelle** and **Hugo**, **Mojtaba**, **Olaf**, **Paulina**, **Sem**, **Soumya**, **Sylvia**, and **Raka**, we had nice and fun daily interactions in the hallways, during coffee breaks and lunch (#Kroketjesbende), but also shared amazing activities including the yearly lab outing, WE-day, barbecuing by the Rhine, 5th of May celebrations, and the annual Christmas High Tea. I cherish the amazing PhD weekends (#ColleaguesWithoutBorders) and yearly festival visits (#Krocketjesbende). What happens at Rock Werchter stays at Rock Werchter.

I would like to extend my gratitude to my current colleagues at the Biomimetics group of the University of Groningen. In particular, I would like to thank you, **Eize**, for your trust and support. I look forward to continuing working with you and the group!

Next to work-related friends, I met numerous other people along the way that helped keeping me sane. In Arnhem, **Iris**, **Roel**, and **Murat**, special thanks for your hospitality and great times! **Iris**, mijn oud-huisgenoot in Groningen, bedankt voor de gezellige avonden!

Mijn buren in Wageningen zijn ook belangrijk geweest. **Jeroen**, we zijn goed bevriend sinds onze studiereis naar Schotland. Via jou ben ik op de Herenstraat terecht gekomen. Mooie avonden in de Zaaier, lekker koken, BBQ'en in de tuin, CoD, klussen, muziek luisteren, varen met de boot, duiken, huisfeestjes en chillen op je werkplaats zijn slechts een aantal leuke dingen die we samen gedaan hebben. Ik wens jou en **Tata** het allerbeste en ik kom sowieso nog weleens over de vloer! Daarnaast wil ik **Manouk** bedanken voor de gezellige en leuke tijd en het verbreden van mijn horizon wat betreft sociaal-maatschappelijk zaken! Samen met andere buren **Marjolein**, **Rutger**, **Jantien**, **Gerlo**, **Luc**, **Rosita**, **Mark**, **Aron** en **Nomy** hadden we met het huis geregeld mooie feesten en vreetfestijnen. Ik ben inmiddels een jaar weg maar ik denk vaak terug aan de gouden tijden op de Herenstraat!

Al jaren belangrijk zijn mijn maten van de middelbare school, **Corné**, **Eric**, **Stephan**, **Roderick** en **Jeroen**. Ik heb Rotterdam (helaas) verlaten maar uit het oog is absoluut niet uit het hart. Door jullie kon ik zaken beter in perspectief plaatsen. Naast dat jullie mij hielpen om dingen te relativeren, hebben we ook zeer veel gelachen en mooie dingen beleefd. Onvergetelijke stedentrips evenals jullie bezoek aan Oostende en Wageningen. Ik ben altijd welkom bij jullie voor een bakkie en verdere escalatie. Ik wens jullie en jullie vriendinnen het allerbeste toe! Gozers, ik hoop dat we nog lang goed bevriend blijven en dat we nog veel moois mogen meemaken!

Nog dichter bij huis wil ik **Rosa** en **Kazuma** bedanken voor hun steun en gezelligheid de afgelopen jaren. Rosa, bedankt dat je er altijd voor mij bent. Je bent het liefste en beste zusje van de wereld! Kazuma, in het bijzonder ontzettend bedankt voor de prachtige illustraties die mijn proefwerk sieren!

Tot slot, wil ik **mam** en **pap** bedanken voor jullie nooit aflatende steun en toeverlaat! Ik waardeer het dat jullie mij altijd vrij hebben gelaten in mijn keuzes waardoor ik altijd heb kunnen doen wat ik leuk vond. De vakanties met jullie in de natuur evenals de escalatie met aquaria in huis hebben zeker mijn nieuwsgierigheid geprikkeld en bijgedragen aan mijn fascinatie voor het leven op aarde. Bedankt voor jullie goede zorgen de afgelopen jaren: van helpen met verhuizen tot aan lekker eten en een warme vertrouwde plek als ik in Rotterdam ben. Jullie zijn de beste ouders van de wereld!

Once again, please allow me to say another huge **thank you** to everyone who has been part of my PhD journey. Your impact will stay with me forever.

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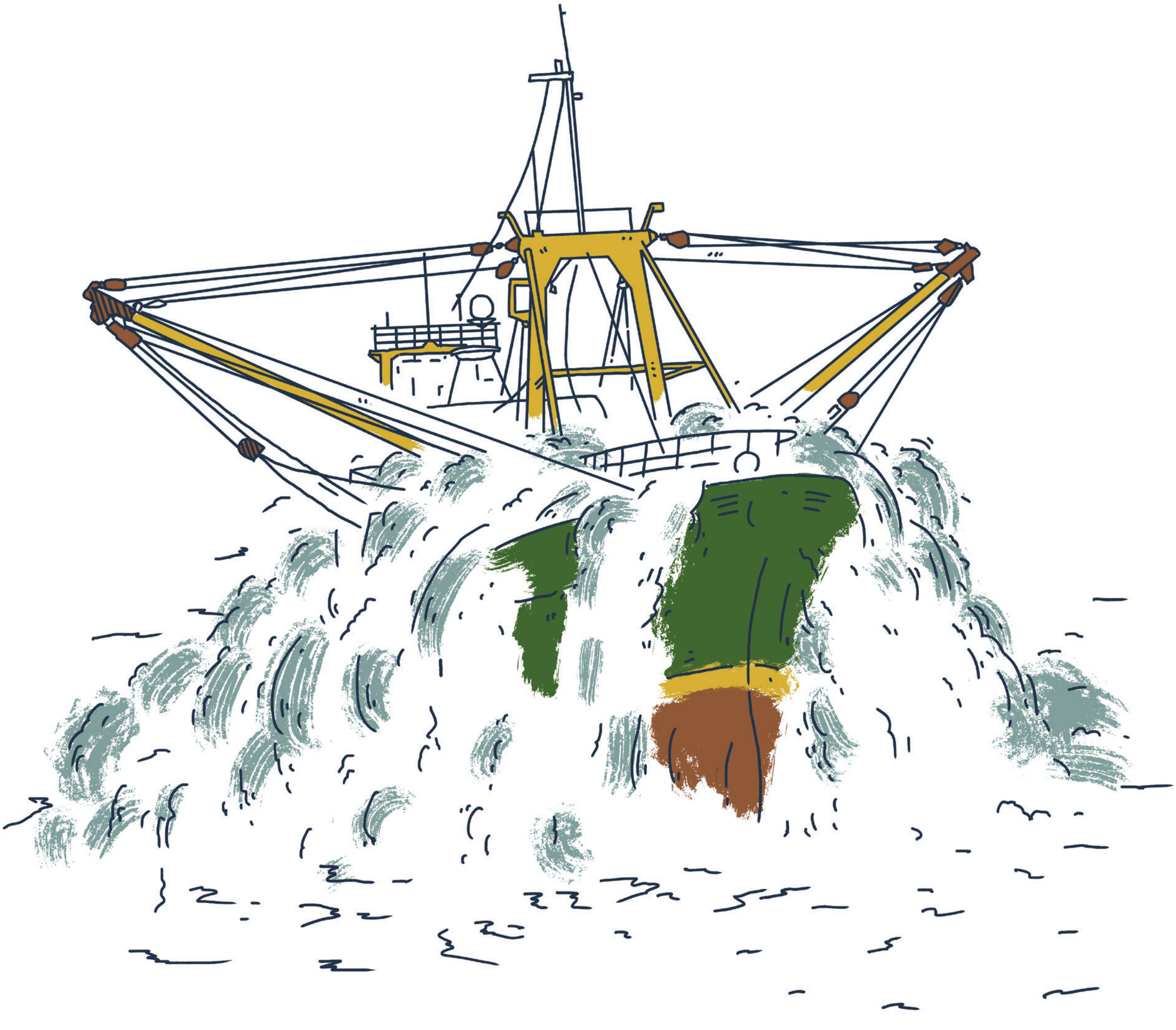

About the author
List of publications
Training and educational activities

About the author

Pim Gabriël Boute was born on 23 August 1991 in Rotterdam, the Netherlands. For his 5th birthday, his parents gifted him an aquarium. His interests in the underwater world have determined many of his life choices ever since.

Pim's interest in aquatic life, and fish in particular, deepened during his high-school period at the Erasmiaans Gymnasium in Rotterdam. His enthusiasm was enhanced by working in an ornamental fish store which resulted in an increasing number of aquaria being brought into the house. For his high-school research project, he and a friend built a Kelvin water dropper – an electrostatic generator – which sparked his interest in electromagnetism.

Due to his fascination with (aquatic) life on Earth, Pim enrolled in 2009 in the BSc programme Biology at Utrecht University, the Netherlands. Here, he participated in the two-year, extracurricular honours program. He conducted his BSc thesis at the Reproductive Biology Lab under supervision of prof. dr. Rüdiger W. Schulz. He studied the effect of photoperiod and nutrition on the gonad development of Atlantic salmon (*Salmo salar*) in aquaculture using histological techniques and microscopy. For his side-job, Pim started working at the aquarium department of a large pet store. He also volunteered at the Atlantic Whale Foundation in Tenerife, Spain. While contributing to ongoing research and informing tourists about cetaceans, he obtained his basic SCUBA diving certification. Pim acquired his Bachelor of Science degree in Biology from Utrecht University in 2012.

With a broad foundation in biology, Pim started the MSc programme Marine Biology at the University of Groningen, the Netherlands, in 2012. His first research project focused on the swimming performance of boxfishes at the Department of Ocean Ecosystems, supervised by prof. (em.) dr. ir. Eize J. Stamhuis. Pim studied the modulation of yaw by the caudal fin in the yellow boxfish (*Ostracion cubicus*). His findings have been published in the peer-reviewed scientific journal *Royal Society Open Science* in collaboration with prof. dr. Sam Van Wassenbergh of the University of Antwerp, Belgium. In addition to the laboratory work on boxfishes, Pim joined GEOTRACES, an international research programme on biogeochemical cycles in the oceans. Under supervision of dr. Willem H. van de Poll, he performed field work on board the RV Pelagia of the Royal Netherlands Institute for Sea Research on the Mediterranean Sea. Pim participated in the first leg of the scientific research cruise between Lisbon in Portugal and Istanbul in Turkey, led by dr. Micha J.A. Rijkenberg. His main responsibility was to collect samples for determining phytoplankton biomass, community structure, and group-specific primary production. The findings were published in the peer-reviewed scientific journal *Marine Chemistry*. In addition to courses at the University of Groningen, Pim took a course on tropical reef ecology at the University of Amsterdam which included a field excursion to Curaçao. Here, Pim obtained his advanced SCUBA certification, followed by the rescue diver certification in the Netherlands. During his master's, Pim also followed courses on aquaculture and fisheries at Wageningen University & Research, the Netherlands. Together with a friend, he joined conventional beam-trawl fishers on their trip on the North Sea. These activities raised his interest in fisheries science, a multidisciplinary research field which involves both environmental and socio-economic aspects. That is why his second research project focused on tuna fisheries in Indonesia. He was supervised by ir. Paul A.M. van Zwieten from Wageningen University & Research and Momo Kochen MSc. from Yayasan Masyarakat dan Perikanan Indonesia. Pim characterised the small-scale tuna fisheries on the islands of Lombok and Buru and performed a total effort and catch estimation to increase clarity on the extent of these fisheries. Pim obtained several grants including the Groninger University Fund travel grant for master's students, the Marco Polo Fund grant for studying abroad, and the Groninger University Fund travel grant for excellent master's students. Pim acquired his Master of Science degree in Marine Biology from the University of Groningen in 2016 (*cum laude*).

In 2016, Pim started his PhD trajectory at the Experimental Zoology Group of Wageningen University & Research. In his PhD project, Pim studied the effects of electrical stimulation on marine organisms in the context of pulse trawling for common sole (*Solea solea*), supervised by dr. ir. Martin J. Lankheet, prof. dr. Adriaan D. Rijnsdorp, and prof. dr. ir. Johan L. van Leeuwen. With his research, Pim aimed to (i) contribute to the mechanistic understanding of the effects of pulsed electric fields on marine organisms by studying responses of benthic invertebrates and fishes to electrical stimulation and (ii) translate these findings into insights useful to assess the impact of flatfish electrotrawling on marine organisms. The obtained insights are used in an interdisciplinary, collaborative research consortium, the "Impact Assessment Pulse-trawl Fishery" (IAPF) project (Chapter 1). Pim acquired his Article 9 of the Experiments on Animals Act certification to qualify as researcher in the design and implementation of laboratory animal testing with fishes. Pim established partnerships with Wageningen Marine Research, the Flanders Research Institute for Agriculture, Fisheries and Food, and the Royal Netherlands Institute for Sea Research. He worked as guest researcher for three months at the Flanders Research Institute for Agriculture, Fisheries and Food in Ostend, Belgium, where he also joined the RV Simon Stevin to collect experimental animals. Furthermore, he joined the FRV Solea with an international team led by dr. Daniel Stepputtis of the Thünen Institute of Baltic Sea Fisheries in Germany. Here, Pim contributed to a selectivity study of an experimentally-modified shrimp pulse trawl. In addition, Pim participated in two commercial flatfish pulse trawler trips to collect fishes for his PhD project and to contribute to ongoing research into the survival of undersized flatfishes and rays. He presented his PhD project findings at the annual meeting of the Working Group on Electrical Trawling (WGELECTRA) of the International Council for the Exploration of the Sea (ICES) and to the wider scientific community at (international) conferences. Pim acquired the Wageningen Institute of Animal Sciences PhD fellowship for research abroad and the ICES Early Career Scientist Support grant for joining the World Fisheries Congress 2021. The findings of his PhD research are presented in this thesis, entitled *Effects of electrical stimulation on marine organisms*.

Next to research, Pim developed an interest in teaching during his PhD project. He supervised in total 13 students doing their thesis or internship, assisted in teaching of the BSc course Marine Life and MSc course Life History of Aquatic Organisms, and gave guest lectures at the HAS University of Applied Sciences. Pim communicated his research and findings to the wider audience through various media channels. Moreover, he joined several courses to expand his disciplinary knowledge and to acquire further competences in research and management.

Since 2021, Pim works as a fellow in teaching and research at the Biomimetics group at the Faculty of Science and Engineering of the University of Groningen. His research focuses on boxfish swimming and suspension-feeding mechanisms of crustaceans and their biomimetic potential and applications. Pim is a lecturer in the field of marine biology, for which he has also started the University Teaching Qualification programme. His teaching revolves around a variety of topics, including zooplankton, nekton, fish biology, secondary production, and fisheries. He also participates in individual teaching by supervising research projects, essays, and colloquia.

In his spare time, Pim enjoys, amongst others, SCUBA diving, an occasional beer with friends, taxidermy, and ornamental fishkeeping.

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Boute, P.G., Tiano J.C., Rijnsdorp, A.D., Lankheet, M.J. (2017). Fishing with electricity / Vissen met stroom. Wageningen World, pp. 18–21. Expert comment in Wageningen University & Research magazine (written by Tessa Louwerens) (in English / Dutch).

Boute, P.G., Tiano J.C., Rijnsdorp, A.D., Lankheet, M.J. (2017). Is pulse fishing the future? / Heeft pulsvissen de toekomst? Wageningen Resource 11(18), pp. 12–15. Expert comment in Wageningen University & Research magazine (written by Tessa Louwerens) (in English / Dutch).

Training and educational activities

Completion of the training and educational activities is in fulfilment of the requirements for the education certificate of the Graduate School Wageningen Institute of Animal Sciences (WIAS). The overview below is based on the European Credit Transfer System (ECTS). One ECTS equals a study load of 28 hours. Some activity categories have a maximum number of ECTS acknowledged by WIAS. A hyphen indicates the instances where this maximum was exceeded and no ECTS were counted.

Activity	*Year*	*ECTS*
The basic package		**3.0**
WIAS Introduction Day	2016	0.3
WIAS course: Essential Skills	2017	1.2
Course: Scientific Integrity & Ethics in Animal Sciences	2018	1.5
Disciplinary competences		**16.3**
Course: INF–22306 Programming in Python	2016	6
PhD Research Proposal	2017	6
Course: Introduction to Laboratory Animal Science (Article 9)	2017	3
Course: Species-Specific Laboratory Animal Science – Fish (Article 9)	2018	0.6
Course: Introduction to R for Statistical Analysis	2019	0.6
Course: Laser Worker Safety	2021	0.1
Professional competences		**6.1**
Course: Supervising BSc and MSc Thesis Students	2017	0.6
WIAS course: High-Impact Writing in Science	2019	1.3
WIAS course: Survival Guide to Peer Review	2019	0.3
Course: Scientific Writing	2019	1.8
Course: Project & Time Management	2020	1.5
WIAS course: The Final Touch – Writing the General Introduction and Discussion	2020	0.6

Activity	*Year*	*ECTS*
Societal relevance		**6**
Is pulse fishing the future? / Heeft pulsvissen de toekomst? Wageningen Resource 11(18), pp. 12–15. Expert comment in Wageningen University & Research magazine (written by Tessa Louwerens) (in English / Dutch)	2017	1
Fishing with electricity / Vissen met stroom. Wageningen World 2, pp. 18–21. Expert comment in Wageningen University & Research magazine (written by Tessa Louwerens) (in English / Dutch)	2017	-
Wat doen elektrische pulsen met vis? Visserijtechniek & Innovatie Special, Visserijnieuws, pp. 18–21. Expert comment in Dutch fishery magazine (written by Gerrit Hakvoort)	2017	0.5
WIAS course: Societal Impact of Your Research	2018	1.5
Europarlementariërs willen pulsvisserij verbieden door fakenews. EenVandaag, AVROTROS, NPO. Expert comment on Dutch television	2018	-
Pulsvissen / De toekomst van de Urker pulsvissers. Nieuw Licht, EO, NPO. Expert comment on Dutch television	2019	1
Visit by Member of the European Parliament. Wageningen, the Netherlands	2019	1
How the World's Squarest Fish Gets Around. Science Trilobites section, The New York Times. Interview in international newspaper (written by Cara Giaimo)	2020	1
De Nederlandse Pim haalde The New York Times met zijn onderzoek naar koffervissen. 5 Uur Live, RTL 4, RTL Nederland. Expert comment on Dutch television	2020	-
Promoveren als basis voor wetenschappelijke carrière. Interview for studiekeuze-kind blog of Wageningen University & Research (written by Hermien Miltenburg)	2020	-
Box-shaped fish. Science World magazine, Scholastic. Expert comment in educational magazine for students (written by Carolyn Malkin)	2020	-
Box-shaped fish. Super Science magazine, Scholastic. Expert comment in educational magazine for kids (written by Carolyn Malkin)	2021	-
Presentation skills		**4**
ICES Working Group on Electrical Trawling 2017. Oral presentation. IJmuiden, the Netherlands	2017	-
WIAS Science Day 2017. Poster presentation. Wageningen, the Netherlands	2017	1
Landelijke innovatiedag vis- en aquacultuur. Poster presentation. Amsterdam, the Netherlands	2017	-
ICES Working Group on Electrical Trawling 2018. Oral presentation. IJmuiden, the Netherlands	2018	-
ICES Annual Science Conference 2018. Oral presentation. Hamburg, Germany	2018	1
Flanders Research Institute for Agriculture, Fisheries and Food. Oral presentation. Ostend, Belgium	2018	-
Scientific meeting Aquaculture and Fisheries Group of Wageningen University & Research. Oral presentation. Wageningen, the Netherlands	2019	-
ICES Working Group on Electrical Trawling 2019. Oral presentation. Ghent, Belgium	2019	-
WIAS Annual Conference 2020. Oral presentation. Lunteren, the Netherlands	2020	1
ICES Working Group on Electrical Trawling 2020. Oral presentation. Videoconference	2020	-
Society for Experimental Biology Annual Conference 2021. Oral presentation. Videoconference	2021	-
World Fisheries Congress 2021. Oral presentation. Videoconference via Adelaide, Australia	2021	1
ICES Working Group on Electrical Trawling 2021. Oral presentation. Videoconference	2021	-

Activity	*Year*	*ECTS*
Teaching competences		**6**
Lecturing		
Guest lecture pitch during course EZO-22806 Marine Life (Wageningen)	2017, 2018	-
Guest lecture at HAS University of Applied Sciences ('s-Hertogenbosch)	2018, 2019	-
Lectures during course WMMB003-05 Principles of Biological Oceanography (Groningen)	2021	-
Guest lectures during course WMEE003-05 Sustainable Use of Ecosystems (Groningen)	2021	-
Lecture during course WBBY044-05 Marine Biology (Groningen)	2022	-
Supervising tutorials and practicals		
Tutorials and practicals on Mollusca and fish swimming during course AFI-31306 Life History of Aquatic Organisms (Wageningen)	2016, 2017, 2019	-
Invertebrate practicals during course EZO-22806 Marine Life (Wageningen)	2017, 2018	-
Practicals on zooplankton and nekton, and excursion to Lauwersoog during course WMMB003-05 Principles of Biological Oceanography (Groningen)	2021	-
Practicals on squid and the Dutch marine flora and fauna during course WBBY044-05 Marine Biology (Groningen)	2022	-
Supervising students		
MSc thesis Raoul Kleppe	2016–2018	2
MSc thesis W. Sarina M. Versteeg	2017	2
BSc thesis Lisanne van Harten	2017	1
BSc thesis Jasper Bleijenberg	2017–2018	1
HBO BSc thesis Mickey Boässon	2018	-
HBO BSc thesis Amerik Schuitemaker	2018	-
HBO BSc internship Clarice Hoogervorst	2018	-
HBO BSc internship Jesse Hoppenbrouwers	2018	-
HBO BSc internship J. Adrian Reid Navarro	2018–2019	-
MSc thesis Marleen E.J. Verdaasdonk	2018–2019	-
HBO BSc internship Lara van den Oever	2019	-
HBO BSc thesis Joey Portier (in collaboration with Justin C. Tiano from the Royal Netherlands Institute for Sea Research)	2019	-
HBO BSc thesis Koen Smid	2019–2020	-
Total		**41.4**

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