

MANAGEMENT BRIEF

No Injuries in European Sea Bass Tetanized by Pulse Stimulation Used in Electrotrawling

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Abstract

Electrotrawling using electric pulse stimulation is a promising alternative to beam trawling in the brown shrimp *Crangon crangon* and Dover Sole *Solea solea* (also known as *Solea vulgaris*) fisheries of the North Sea. In the sole fishery, a 40–80-Hz pulse stimulation induces tetany in the muscles, which may result in injuries. Whereas no injuries have been reported in flatfish or selachian sharks and rays, electrically induced spinal injuries have been observed in gadoids such as Atlantic Cod *Gadus morhua* and Whiting (also known as European Whiting) *Merlangius merlangus*. This may indicate that fish species with a fusiform shape are more susceptible to electric pulses. Similar variation among species in electrically

induced spinal injuries has been observed in freshwater electrofishing, although large variability in vulnerability has been reported among different freshwater fusiform species. Therefore, we aimed to assess the vulnerability of another, nongadoid, fusiform osteichthyan: Sea Bass *Dicentrarchus labrax* (also known as European Bass *Morone labrax*). Two length groups of Sea Bass (31.3 ± 2.2 and 42.1 ± 2.5 cm) were exposed to electric pulses as used in commercial electrotrawls targeting Sole (80 bipolar pulses per second, 2% duty cycle). Thereafter, the fish were monitored daily and then euthanized 14 d after exposure for gross, radiographic, and histologic examination. No injuries were found in fish exposed to the electrical pulses. Differences in vertebral morphology among fusiform species may result in varying vulnerabilities to electrically induced

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Received April 24, 2017; accepted November 22, 2017

spinal injuries. As a result, electrically induced spinal injuries and/or their variability in both marine and freshwater species may be determined by similar morphological parameters.

Electrotrawling with electrical pulses is a very promising alternative to conventional beam trawling, particularly for North Sea fisheries targeting Dover Sole *Solea solea* (also known as *Solea vulgaris*) and brown shrimp *Crangon crangon*. In these so-called “electric pulse trawls,” the conventional mechanical stimulation by tickler chains or chain matrices is replaced by electrodes generating an electrical stimulus that causes tetany (Soetaert et al. 2015). The switch from mechanical to electrical stimulation, combined with a lower towing speed that results in a smaller area being trawled, offers four major advantages: fuel savings up to 50% (van Marlen et al. 2014), reduced physical impact on the sea bottom (Depestele et al. 2015), 16–80% reductions in benthos discards (Rasenberg et al. 2013; van Marlen et al. 2014), and a 30–50% decrease in the capture of undersized fish (Rasenberg et al. 2013; van Marlen et al. 2014).

However, concerns have been raised regarding possible negative side effects of these electric pulses on marine organisms, especially since spinal injuries have been observed in gadoids, particularly Atlantic Cod *Gadus morhua* (van Marlen et al. 2014; de Haan et al. 2016; Soetaert et al. 2016a, 2016b, 2016c). Nevertheless, tetany did not induce severe lesions in flatfish such as Sole (Soetaert et al. 2016b), Dab *Limanda limanda* (also known as *Pleuronectes limanda*; de Haan et al. 2015), or Small-Spotted Cat Sharks (also known as Spotted Dogfish) *Scyliorhinus canicula* (Desender et al. 2017). Based on these results, it seems that osteichthyan fusiform fish are especially vulnerable to injury by electric pulses.

Electrofishing in freshwater makes use of entirely different pulse settings and equipment and aims for a different reaction, i.e., involuntary movement toward the electrode (electrotaxis), which may result in tetany or narcosis. Unfortunately, strong tetany of the muscles and associated spinal injuries are a common and well known side effect (reviewed by Snyder 2003), although vulnerability can vary widely both within and among species. The latter even includes large differences among various families with a fusiform body shape, ranging from no or minor injuries in carp (family Cyprinidae) and bass (family Centrarchidae) to very high spinal injury rates in trout, char, and salmon (Salmoninae). This suggests that although vulnerability is partially rooted in morphology, factors other than fish musculature (such as the number of vertebrae) need to be considered when evaluating the effect of electric pulses. At present, no experiments have been performed with marine fusiform osteichthyans other than Atlantic Cod, making it impossible to validate this

hypothesis for nongadoid marine fusiform species. Therefore, the aim of the present study was to assess the impact of the tetanizing electric pulses used in electrotrawls targeting Sole on Sea Bass *Dicentrarchus labrax* (also known as European Bass *Morone labrax*), which also inhabit the North Sea and thus may be exposed to electric pulses.

METHODS

We obtained 44 Sea Bass from a commercial farm (Ecloserie Marine de Gravelines, France); they were acclimated for 4 months, fed three times a week (Marico Supreme 16, Coppens International), and divided into two size-groups (group 1: 29 fish, 31.3 ± 2.2 cm [mean \pm SD]; group 2: 15 fish, 42.1 ± 2.5 cm). All fish were housed in two tanks 2.75 m long \times 1.00 m wide \times 1.20 m high filled with natural seawater in a recirculation system with a common matured and fully functional biological filter. The water depth was 0.9 m and a 12 h light : 12 h dark photoperiod was used. The experiments were approved by the Animal Welfare Ethical Committee of the Institute for Agricultural and Fisheries Research (ID 2011/170).

The Sea Bass were positioned between two wire-shaped electrodes which consisted of two copper conductors (0.18 m, 26 mm in diameter) lifted from the bottom by two polyvinyl chloride (PVC) discs (10 mm width, 70 mm in diameter) at both ends and separated by an insulator of 0.57 m. Therefore, they were placed with their longitudinal body axis as close to the conductor as possible, while the tip of their snout was located at the front of the first conductor (Figure 1). This was done by holding the fish in a triangular V-shaped cage made of PVC netting as described by de Haan et al. (2016). A 60-V potential difference over the electrodes was applied by a laboratory pulse generator (LPG1, EPLG, Bruges, Belgium) capable of reaching a maximum output of 150 V, 280 A, and 42 kW. The generator was equipped with a feedback system to ensure that the output exactly matched the values set. Additionally, the output was double-checked using an oscilloscope (Tektronix TDS 1001B). Sixty V was chosen because this is the upper value achievable in commercial electrotrawls targeting Sole and was used in previous studies (Soetaert et al. 2016a, 2016d). Pulse settings similar to those applied by commercial electric pulse trawls targeting Sole were used: a 60-V bipolar stimulation of rectangular shaped pulses with a duration of 0.25 ms and a frequency of 40 Hz, resulting in 80 electrical pulses per second (Figure 2). This setting results in a duty cycle of 2%, i.e., 80 pulses of 0.25 ms per 1,000 ms. The exposure duration was set to 2 s.

All fish were transferred individually with a dip net from the housing tanks to the exposure tank. Fish in group 1 ($n = 20$) and group 2 ($n = 11$) were exposed according to the same protocol described in Soetaert et al. (2016a). Briefly, each fish was held in a PVC net near the electrode and gently pushed down to hold it near the electrode. When the fish

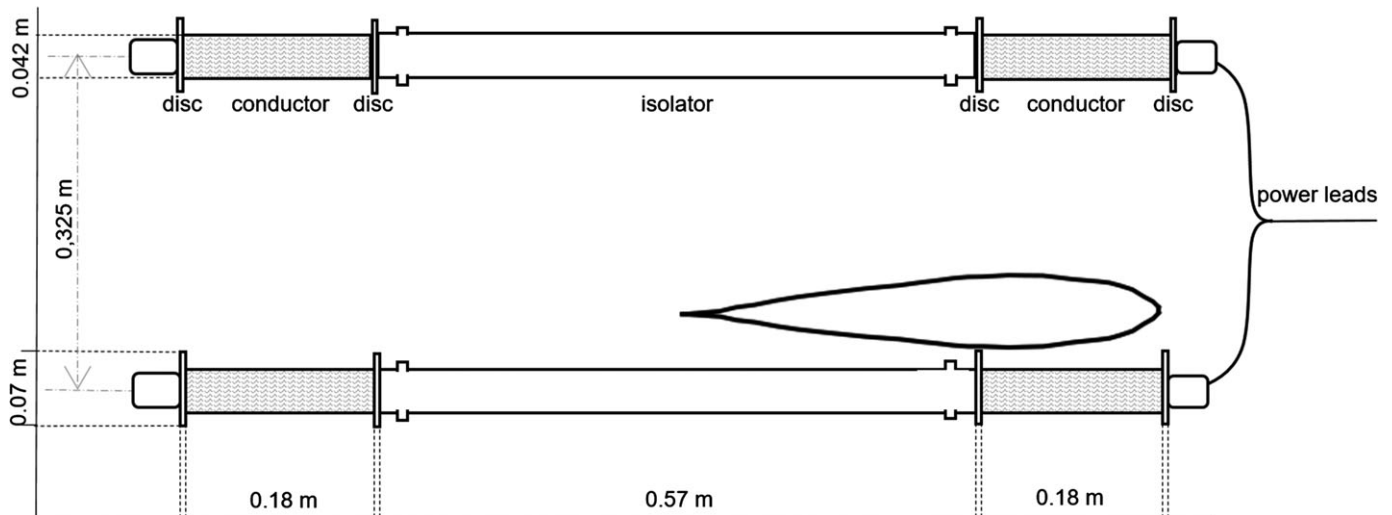


FIGURE 1. Schematic top view of the principal setup in which Sea Bass were exposed to electric pulses. The dashed lines indicate the size of each part.

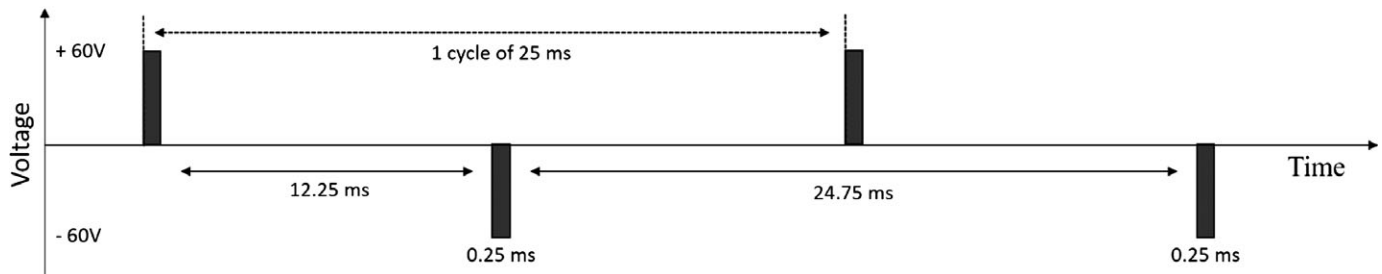


FIGURE 2. Illustration of the pulse stimulation to which Sea Bass were exposed for 2 s: a 60-V stimulation of 80 bipolar and rectangular shaped pulses per second, each pulse having a duration of 0.25 ms. This results in a frequency of 40 Hz, i.e., 40 identical bipolar cycles of 25 ms.

could not move and was properly positioned parallel to the electrodes, it was exposed to the electric stimulus. All fish showed tetany, and once this was observed the fish were allowed to move rather than being forced to remain in their initial position. Then, the fish were held in the netting material for 15 s, tagged (Floy tag) in the first dorsal fin, and transferred to their housing tanks. The remaining fish (9 in group 1, 4 in group 2) were treated similarly but were not exposed and served as controls. All fish were monitored daily, fed three times a week and sacrificed 2 weeks later. Then they were necropsied and examined for external and internal lesions, particularly spinal injuries that might have occurred during tetany. Their length, total weight, and somatic weight (eviscerated fish [W_s]) were recorded. The W_s values were used to calculate Fulton's condition factor (K) for each fish, i.e.,

$$K = 100 \cdot W_s / L^3,$$

where L is length (Bagenal 1978). Additionally, internal organs were examined for lesions, and samples of gill,

dorsal muscle (base of the third dorsal fin), heart, liver, spleen, gut, and kidney were collected and processed for histological examination and comparison with the control animals, as described in Soetaert et al. (2016a). After necropsy, fish carcasses were labelled and frozen, and lateral and dorsoventral radiographs with X-rays (60 kV, 12.5 mAs) were taken at Ghent University (EDR6 CANON, type XCDI-50G, flat panel detector; scintillator and amorphous silicon sensor LANMIT 4, Santa Clara, California). All photographs were examined to detect possible malformations, fractures, or luxations, and the number of vertebrae was determined.

RESULTS

When transferred to the exposure tank, fish exhibited slow swimming behavior in the netting material, mostly pressing their noses to the ends of the net. Immediately after initiation of the electric pulses, all exposed Sea Bass showed tetany. In all but one fish from each group, this was accompanied by distended opercula over the entire exposure time. No

bending of the body was observed. In the first seconds following exposure, all Sea Bass exhibited an escape reaction and swam away from the point of exposure. This reaction varied between a short (2 s) of swimming behavior at low or moderate speed (<1 tail beat per second) to a more intense 5 s of agitated swimming, during which some fish tried to jump out of the netting material. Control fish behaved the same as exposed fish during and after restraint but did not exhibit any escape behavior. When released into the housing tanks, all fish returned to their normal swimming behavior.

During the 2-week observation period, none of the fish died and all demonstrated normal feeding behavior. At necropsy, no external or internal abnormalities were found. All Sea Bass had a full stomach after being fed 24 h prior to sacrifice. The results with respect to length, total weight, and somatic weight are presented in Table 1. X-ray analysis did not reveal any spinal injuries or acute lesions, but in 15% of the fish congenital defects such as compressed vertebrae, chronic fractures, and block vertebrae were found (Figure 3). Finally, histological examination did not reveal any abnormalities in the gills, heart, dorsal muscle, gut, spleen, kidney, or liver.

DISCUSSION

The major aim of this study was to investigate the vulnerability of Sea Bass to electrically induced injuries and to reveal differences in susceptibility among fish species. Exposed fish exhibited tetany on being electrically stimulated, followed by an escape response similar to that reported for Atlantic Cod (de Haan et al. 2016; Soetaert et al. 2016a, 2016b); marine flatfish such as Sole, Dab, and Plaice *Pleuronectes platessa* (Stewart 1977; Soetaert et al. 2016b); and invertebrates such as brown shrimp *C. crangon* (Soetaert et al. 2014, 2016d).

In the present study, Sea Bass were exposed right near the electrode, where the highest field strengths (37–155 V/m) can be found (de Haan et al. 2016). Nevertheless, no adverse side effects were observed. This accords with the absence of injuries in Sea Bass of 10 and 30 cm exposed

to much weaker field strengths (9.4–15.1 V/m)—and thus having much smaller potential for causing harm—in an electrode setup typically used in freshwater electrofishing (D’Agaro and Stravisi 2009). In addition, the histopathological results of the present study did not reveal any microscopic lesions on the gills, muscles, or internal organs, indicating either that no lesions occurred or that they had healed within 2 weeks of exposure. Although the results of this laboratory study are reassuring, caution is still warranted because there may be large variability in vulnerability among fish of different origins (Soetaert et al. 2016a) and a few, statistically significant electrically induced spinal injuries have been observed in freshwater Centrarchidae (Snyder 2003).

The absence of spinal injuries in Sea Bass in this and previous studies stands in contrast to the negative effects observed in gadoids such as Atlantic Cod and Whiting (also known as European Whiting) *Merlangius merlangus*. Therefore, we postulate that the large interspecies difference in vulnerability to electrically induced injuries observed in freshwater electrofishing may also apply to marine fishes. The differences be caused by a similar set of decisive morphological parameters. First, although all exposed fish clearly demonstrated tetany, indicating that the electric field was able to cause a physical response, the thick ctenoid scales of Sea Bass might shield the electric field more efficiently than the thin cycloid scales of Atlantic Cod, resulting in lower penetration into the fish’s body and fewer effects. Second, the difference in vertebral injuries may be related to differences in number of vertebrae, as previously suggested by Soetaert et al. (2015). The lower number (23–25) and thus larger vertebrae of the Sea Bass in the present study contrast with the high number (51–54) of smaller vertebrae of gadoids such as Atlantic Cod (Soetaert et al. 2016a) and Whiting (51–53; Milic and Kraljevic 2011). The possible importance of vertebral morphology as factor in the vulnerability for spinal injuries is further emphasized by the occurrence of electric stunning-induced spinal injuries in Pollock *Pollachius virens* (52–55 vertebrae) and Atlantic Herring *Clupea harengus* (53–58

TABLE 1. Means \pm SDs of different physiological parameters at postmortem examination and range of vertebrae counts for small (group 1) and large (group 2) Sea Bass.

Parameter	Group 1		Group 2	
	Control	Exposed	Control	Exposed
Number of fish	9	20	4	11
Size (cm)	31.1 \pm 2.1	31.4 \pm 2.2	39.5 \pm 2.9	43.0 \pm 1.5
Weight (g)	331.4 \pm 57.9	337.7 \pm 72.6	718.1 \pm 180.1	905.1 \pm 105.0
Somatic weight (g)	304.4 \pm 53.8	306.3 \pm 72.6	641.2 \pm 164.0	808.1 \pm 91.0
Fulton’s <i>K</i>	1.00 \pm 0.05	0.98 \pm 0.10	1.02 \pm 0.06	1.02 \pm 0.11
Number of vertebrae	24–25	24–25	24–25	23–25

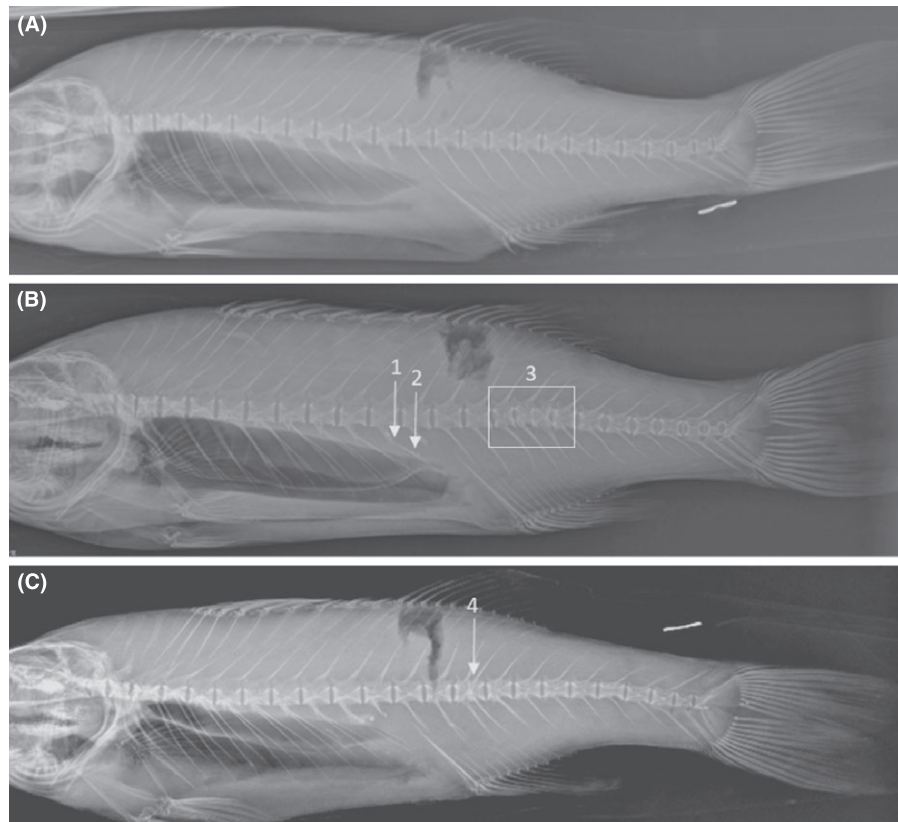


FIGURE 3. X-rays of (A) a normal large Sea Bass and (B) large and (C) small Sea Bass with congenital and/or chronic deformities: 1 = chronic rib fracture, 2 = multiple new bone formation, 3 = compression of vertebrae with mild secondary degenerative changes at the dorsal aspect, and 4 = a block vertebrae. The dark blotches at the base of the final dorsal fins were caused by the sampling of muscle tissue for further histological examination. No recent vertebral or spinal injuries resulting from electric-induced tetany were found.

vertebrae) (Roth et al. 2004; Nordgreen et al. 2008). Salmonidae, having a similar morphology as Atlantic Cod, with cycloid scales and a large number of vertebrae (ranging from 55–60 for salmon [Fraser et al. 2015] to 60–66 for trout [Scott and Crossman 1973]), have been reported to be much more susceptible to spinal injuries than other fusiform species such as bass (30–32 vertebrae; Scott and Crossman 1973; Snyder 2003). This suggests that a lower number of larger vertebrae may result in greater mechanical strength and/or robustness of the Sea Bass's vertebral column, making this fish less prone to the development of spinal injuries.

The above hypothesis accounts not only for interspecies differences but also intraspecies variability. Indeed, the most vulnerable (and hence the most studied) species in freshwater and marine electrofishing have shown a highly variable incidence of spinal injuries: 0–78% for Salmonidae (Snyder 2003) and 0–70% for Atlantic Cod (Soetaert et al. 2016a). Although extrapolating from and comparing different studies should be done with great care due to the high variability between the experimental setups as well as in the origin and rearing history of the fish, the similar trends between freshwater and marine electrofishing are important and warrant further investigation.

Indeed, decisive morphological parameters determined through the much more extensive freshwater electrofishing research can be used in a mechanistic framework to help predict which marine species are most vulnerable and thus those on which research should be a priority. The possibility of revealing common underlying mechanisms in future research also offers new opportunities for freshwater and marine electrofishing research toward the common goal of reducing electrically induced injuries.

ACKNOWLEDGMENTS

Maarten Soetaert received a PhD grant from the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT-Vlaanderen). Additional support was given by the Flemish Scientific Institute for Agricultural and Fisheries Research (ILVO), which also paid for the finalization of this paper, and the European Fisheries Fund (EVF). F. Delanghe and D. Vuylsteke are thanked for their assistance in taking care of the animals, M. Vercauteren for her help during autopsies, and C. Puttevels and D. Ameye for embedding and processing the samples. Finally, we thank the reviewers who helped

improve and fine-tune this paper. There is no conflict of interest declared in this article.

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