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Electrosensory Impairment in the Atlantic Stingray, *Hypanus sabinus*, After Crude Oil Exposure

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ABSTRACT

Elasmobranchs are renowned for their extremely sensitive electrosensory system, which is used to detect predators, prey, and mates, and is possibly used for navigation. The proper functioning of the electrosensory system is thus critical to fitness. The objective of this study was to test whether exposure to crude oil impairs the electroreceptive capabilities of elasmobranch fishes. Electrosensory function was quantified from six stingrays before and after exposure to a concentration of oil that mimicked empirically measured concentrations along the coast of Louisiana following the Deepwater Horizon spill. Orientation distance (cm), and angle with respect to the dipole axis of a prey-simulating electric field were used to derive the electric field intensity that elicited a response. Oil exposed stingrays continued to exhibit feeding behavior, but they initiated orientations to prey-simulating electric fields from a significantly closer orientation distance. The mean orientation distance after oil exposure was 5.29 ± 0.41 SE cm compared to a pre-exposure orientation distance of 7.16 ± 0.66 SE cm. Stingrays required a mean electric field intensity of 0.596 ± 0.21 SE μV cm^{-1} to initiate a response after oil exposure, compared to a mean of only 0.127 ± 0.03 SE μV cm^{-1} in uncontaminated seawater. Oil exposed stingrays thus exhibited a response to a stimulus approximately 4.7 times greater than controls. Stingrays impacted by an oil spill appear to experience reduced electrosensory capabilities, which could detrimentally impact fitness. This study is the first to quantify the effects of crude oil on behavioral electrosensory function.

1. Introduction

Electroreception is a sensory modality that has evolved independently in various vertebrate clades including sarcopterygians, chondrosteans, elasmobranchs, teleosts, amphibians, monotremes, and placental mammals (Bullock et al., 1983; Czech-Damal et al., 2012; Crampton, 2019; Newton et al., 2019). This sensory system is used to detect minute bioelectric fields produced by aquatic organisms. Bioelectric fields can vary in magnitude and frequency and consist of direct current (DC) and alternating current (AC) components (Kalmijn, 1972; Bedore and Kajiura, 2013). Ion leakage from the mouth, gills, siphon, and cloaca produce a DC field around an organism's body (Wilkens and Hofmann, 2005). The rhythmic opening and closing of the mouth and gills imposes a time-variable component (Bedore and Kajiura, 2013). The resulting electric field around the organism can be used by electro-sensitive species to locate these potential prey items.

Although electroreception is widespread across various taxa, it is perhaps best known within the elasmobranch fishes (Newton et al., 2019). The elasmobranch electrosensory organ, the ampullae of Lorenzini, is extremely sensitive and allows them to detect electric fields of <5 nV cm^{-1} in seawater (Kalmijn, 1982). The structure of the ampullae of Lorenzini consists of hundreds to thousands of pores on the surface of the skin, each connected by a subdermal tubule that terminates in an ampulla composed of multiple alveolar sacs (Waltman, 1965). The alveolar sacs are lined with a single layer epithelium that contains modified sensory hair cells and support cells (Waltman, 1965; Zakon, 1988). The tubules and ampullae are filled with a highly conductive glycoprotein gel that acts as a low impedance pathway from the seawater to the sensory hair cells (Waltman, 1965; Brown et al., 2002; Josberger et al., 2016). The electrosensory primary afferent neurons projecting from the ampullae of Lorenzini have a regular resting discharge rate in the absence of an electric stimulus (Kantner et al., 1962). The discharge rate increases when the pore is presented with a cathodal stimulus and decreases when presented with an anodal stimulus (Murray, 1962, 1965). Elasmobranchs use their electrosensory system for prey detection (Kalmijn, 1971), mate detection (Tricas et al.,

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1995), predator detection (Sisneros et al., 1998) and possibly to mediate orientation to the earth's magnetic field and hence be used for navigation (Kalmijn, 1982; Paulin, 1995; Newton and Kajiura, 2017).

The pores of the electrosensory organ come into contact with the external environment on the surface of the skin. As a result, environmental pollutants could interact with the glycoprotein gel and impact electrosensory function. Several anthropogenic pollutants are capable of damaging various aquatic sense organs. For example, the lateral line in brook trout becomes hypersensitive after exposure to DDT (Anderson, 1968). Red drum and sheepshead minnow larvae exposed to low levels of weathered crude oil exhibit compromised eye development and reduced visual function (Magnuson et al., 2018). The impact of environmental pollutants has been most widely studied in the olfactory system. Heavy metals have been documented to affect olfactory structure and function in a variety of fishes (Klaprat et al., 1992; Tierney et al., 2010). Similarly, exposure to crude oil from the Deepwater Horizon has been shown to significantly depress olfactory sensitivity in the Atlantic stingray (Cave and Kajiura, 2018). Although other sensory modalities have been documented to be impacted by pollutants such as crude oil, no data exist on the effect of any environmental pollutant on the function of the electroreceptive system of elasmobranchs. If the electrosensory system experiences an impairment similar to that observed with other sensory systems, it could reduce the animal's ability to detect predator or prey bioelectric fields, and thus reduce fitness. Therefore, the objective of this study was to test whether exposure to crude oil from the Deepwater Horizon impacts the electrosensory function of elasmobranch fishes.

2. Materials and Methods

2.1. Preparation

Atlantic stingrays, *Hypanus sabinus* (Lesueur, 1824) were collected from the Indian River Lagoon by the Florida Fish and Wildlife Conservation Commission, and Florida Atlantic University students using a seine net, under Florida Fish and Wildlife Conservation Commission Special Activities License: SAL-12-1413B-SR. Animals were transported from the capture location to the Florida Atlantic University Marine Laboratory at the Gumbo Limbo Environmental Complex (Boca Raton, FL, USA) and acclimated to the ambient seawater. Stingrays were kept in a 244 × 122 cm fiberglass tank supplied with flow-through seawater and were fed thawed shrimp to satiation every other day. All animals were feeding in captivity for a minimum of one week prior to the start of experimentation and all experiments were conducted in accordance with FAU IACUC protocol A13-21.

To determine the toxicological effects of crude oil, stingrays were exposed to a high-energy water accommodated fraction (HEWAF) oil solution. HEWAF stock solution was prepared by blending 4 g of Slick B crude oil in 4 L of seawater (1 g L^{-1}) in a 4 L Waring blender (Waring, CB15) for 30 s. An experimental tank, identical to the holding tank (244 × 122 cm), was filled to a depth of 5 cm (~379 L) with pristine seawater, and 37.9 L of HEWAF stock solution was mixed into the tank. The final concentration of oil in the experimental tank in which the stingrays were exposed was 0.01%. This concentration simulated the level of contamination empirically measured along the Louisiana shoreline following the Deepwater Horizon oil spill (Sammarco et al., 2013; Incardona et al., 2014). For oil exposure treatments, the inflow and outflow valves of the experimental tank were closed, the water was aerated with two air stones, and a stingray was placed into the tank and held in the oily water for 48 h. Water quality and oil concentration were maintained with regular water changes of 114 L of a seawater solution with the same HEWAF concentration every eight hours. This ensured that the concentration of oil in the experimental tank did not change over the duration of the experiment. For control experiments, stingrays were tested under identical conditions in the holding tank with no HEWAF added to the seawater.

2.2. Apparatus

Electrosensory response was tested on six stingrays using a prey-simulating electric field, exercising methods similar to previous studies (Kajiura and Holland, 2002; McGowan and Kajiura, 2009). Trials were conducted in an experimental tank, which had the same dimensions as the holding tank and was located immediately adjacent. The only difference between the two tanks was that the experimental tank had a 213 × 122 cm, white, acrylic plate with two electric dipoles spaced 40 cm apart, placed at the bottom, and this plate covered nearly the entire floor of the tank (Fig. 1). Each dipole consisted of two, 1 mm diameter holes spaced 1 cm apart to simulate a small prey item. One 2.5 cm square by 1 cm thick acrylic block was bonded to the acrylic plate under each dipole. The acrylic block was machined with two separate holes that extended from the holes on the surface of the plate to threaded hose barbs fitted into the block. Flexible tubing was fitted over the two hose barbs and filled with seawater from the tank using a 50 mL syringe. The seawater filled hoses were press fitted snugly to gold-plated stainless steel pins at the end of a two-conductor 18 American Wire Gauge underwater cable (Teledyne Impulse, San Diego, CA). Each cable was connected to an electric stimulator that produced a prey-simulating DC electric field and allowed each dipole to be individually activated (Kajiura and Holland, 2002). A multimeter in series measured the electrical current delivered to the dipoles. Twenty cm diameter circles drawn on the electrode plate around each dipole were used to calibrate the analysis software. A high definition video camera (Sony HDR-CX360) was mounted above the middle of the tank and aimed straight down to film orientations to the active dipoles.

2.3. Protocol

Food was withheld for 48 h (2 days) prior to control and oil-exposure trials to motivate foraging behavior during the experiment. After the starvation period, stingrays were moved from the holding tank into the experimental tank and allowed to acclimate for 20 minutes before initiating the experiment. Shrimp rinse was poured into the tank between both dipoles to initiate foraging behavior, as evidenced by increased swimming velocity and frequency of turning. Video recording commenced when the stingrays started to exhibit foraging behavior, then the stimulator was turned on to deliver an electrical current of ~8.1-8.9 μA to one of the randomly selected dipoles. The other dipole

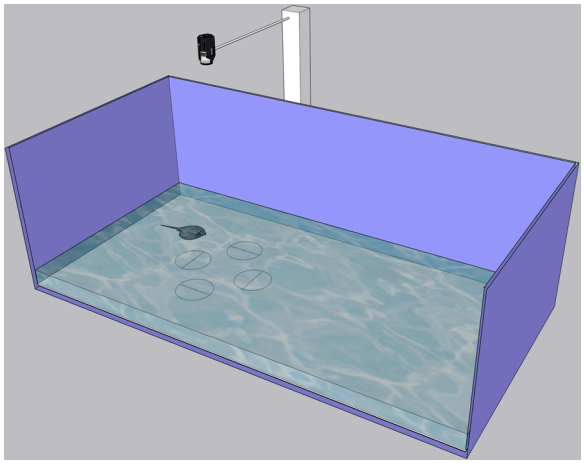

Fig. 1. Experimental setup used to test the response of stingrays to prey-simulating electric fields. The floor of the tank had four electrode pairs, and only two were used in this study. A line was drawn through the dipole axis and a 20 cm diameter circle was drawn around each dipole to provide a scale for the video camera mounted overhead. Stingrays were unable to see the stimulator controls and were thus not provided with any visual cues when the dipoles were activated.

remained off as a procedural control. The electric stimulus remained on until a stingray oriented and bit at the center of the dipole, then the stimulus was turned off and the other dipole was turned on. This procedure was repeated until the stingray no longer bit at the electrodes, or exhibited slower swimming. Duration of each trial ranged between 30-60 minutes and varied among individuals. Stingrays tested in the crude oil treatment exhibited longer trial durations than stingrays tested in the control treatment. Stingrays were fed to satiation after trials were completed. The minimum time between control and oil exposure trials was 72 h (3 days) to account for post-experimental feeding, and subsequent 48 h fasting. The maximum time between control and 48 hour oil exposure trials was 120 hours (5 days). All trials were conducted within 1 hour of the target time.

2.4. Analysis

Video footage of the orientations was downloaded to the computer and viewed using Quicktime Player v10.4 (Apple Inc.). The frame in which a stingray initiated an orientation toward a dipole was exported from Quicktime and imported into ImageJ v1.43 (NIH). The 20 cm diameter circle on the acrylic plate was used to calibrate the ImageJ software. Orientation distance (cm) was measured from the center of the dipole to the posterior margin of the closest spiracle, which approximated the position of the hyoid ampullary cluster. The angle with respect to the dipole axis at which the stingray initiated its orientation toward the dipole was also measured using ImageJ. The orientation distances measured for all responses were averaged for each stingray. A Shapiro-Wilk's test ($p = 0.05$) was applied to determine whether the distance data conformed to the normality assumption and a Levene's test ($p = 0.05$) was applied to test for homoscedasticity.

The distance and angle were used to calculate the electric field at the point of orientation using the equation for charge distribution around an ideal dipole (Griffiths, 1989):

$$E = \cos\theta \frac{\rho I d}{\pi r^3}$$

In this equation, E is the electric field (μV cm^{-1}), ρ is the resistivity of the seawater (Ω cm), I is the applied current (8.1-8.9 μA), d is the gap distance between the two poles of the dipole (1 cm), r is the measured orientation distance (cm) and θ is the measured orientation angle with respect to the dipole axis (deg.). The calculated electric field data were tested for normality with a Shapiro-Wilk's test ($p = 0.05$) and tested for homoscedasticity with a Levene's test ($p = 0.05$). Since the same individuals was tested under both control and oil exposure treatments and assumptions of normality and homoscedasticity were accepted, a paired t-test was used to compare orientation distances between treatments. Because the calculated electric field values failed the normality and homoscedasticity tests, a non-parametric Wilcoxon signed-rank test ($\alpha = 0.05$) was used to compare calculated electric fields at the orientation point between treatments.

The mean number of bites under control and oil exposure were compared using a paired t-test ($\alpha = 0.05$). Swimming velocity was derived from the video footage to determine if the stingrays exhibited similar motivation during searching. The swimming velocity was calculated by measuring the distance traveled over a minimum of 1 second (30 frames) as a stingray swam in a straight line immediately before it initiated an orientation toward the dipole target. The 20 cm diameter circles on the plate were used to calibrate the ImageJ software. The swimming velocity data were tested for normality with a Shapiro-Wilk's test ($P = 0.05$), for homoscedasticity with a Levene's test ($P = 0.05$), and subsequently tested between treatments with a paired t-test. All statistical analyses were conducted using R statistical software package version 3.3.3.

2.5. Electric field measurement

The electric field produced by the stimulator was measured in both uncontaminated seawater and seawater prepared with 100% HEWAF, following methods described previously (Bedore and Kajiura, 2013; Harris et al., 2015). Briefly, an acrylic tank (89 cm × 43 cm × 21 cm) was filled to a depth of 5 cm with seawater to mimic the experimental conditions. An acrylic plate (89 cm x 43 cm) was placed on the bottom of the tank and flexible tubing connected underwater cables to the same electric stimulator used in the experiments. A non-polarizable Ag/AgCl recording electrode mounted on a moveable track was positioned with the electrode tip approximately 5 mm above the surface of the plate. An identical electrode was affixed to the wall in the far corner of the tank with the tip submerged in the seawater. Output from the electrodes was differentially amplified, filtered, digitized, and recorded on the computer using LabChart software (Version 5.5.6, AD Instruments). The electric stimulator produced a prey simulating DC electric field in the water above the plate. The recording electrode was positioned along the dipole axis at various distances (1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 10 cm, 15 cm, 20 cm, and 25 cm) from the dipole center. The order in which voltage was measured at each distance was randomized. The voltage at each distance was measured three times and averaged. A t-test was used to compare voltage at each location for uncontaminated, and HEWAF-treated seawater. The electric field measurements were collected only once to determine if crude oil altered the electric field propagation. To minimize differences in electrical conductivity due to evaporation of aromatic hydrocarbons within the HEWAF solution, all electric field measurements were taken within 2 h of the HEWAF being prepared. Furthermore, to minimize any differences in conductivity due to differences in water temperature, the water used to create the HEWAF in the electric field measurement and used in the behavioral trials was from the same source and at the same temperature.

3. Results

Responses to prey-simulating electric fields were recorded from six stingrays. These individuals were first tested in uncontaminated seawater conditions and then tested again at least 72 hours later after exposure to HEWAF for five hours, and 48 hours. The 48 h exposure treatment was excluded from analysis due to a lack of bites at the dipoles.

The maximum distance at which a stingray oriented to a dipole was 10.07 cm in the control treatment compared to 6.18 cm in the five hour HEWAF treatment. The mean distance at which the stingrays oriented towards the dipole was significantly shorter after HEWAF exposure (Mean 5.29 cm, SE ± 0.41) compared to when they were tested in uncontaminated seawater prior to HEWAF exposure (Mean 7.16 cm, SE ±

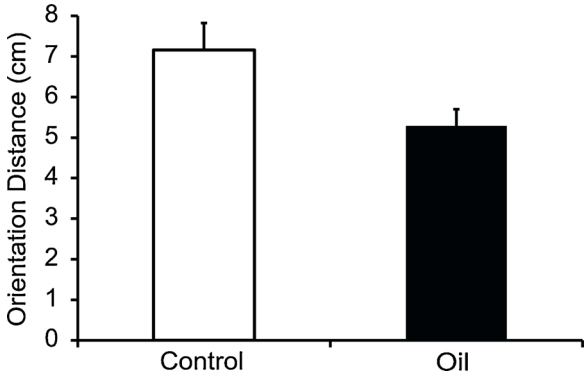

Fig. 2. The mean distance (+SE) at which stingrays initiated their orientation towards the dipole in control and 5 h HEWAF-exposure treatments. HEWAF-exposed stingrays initiated an orientation toward the dipole from a distance significantly closer to the source.

0.66) (Paired t-test, t = 3.8345, P = 0.012) (Fig. 2).

Additionally, the mean electric field intensity that elicited a bite response was significantly greater in HEWAF exposed stingrays compared to when the same individuals were tested under uncontaminated seawater conditions (Fig. 3). In uncontaminated water, stingrays initiated a response at a mean electric field intensity of 0.127 ± 0.03 SE μV cm^{-1}, whereas HEWAF exposed individuals required a mean stimulus of 0.596 ± 0.21 SE μV cm^{-1} to initiate a response. HEWAF exposed stingrays required a stimulus approximately 4.7 times greater, which differed significantly from the controls (Wilcoxon signed-rank test, Z = -2.15, P = 0.031, r = 0.48). The minimum electric field that stingrays were able to detect was weaker under control treatments (0.055 μV cm^{-1}) than under HEWAF treatments (0.161 μV cm^{-1}).

To determine whether these effects were due to impaired physiological capabilities or a lack of motivation, the swimming velocity and the number of bites at the dipole were compared between control and HEWAF treatments. The number of bites did not differ significantly among individuals between control (Mean 10.3 ± 1.86 SE) and five hour HEWAF exposure treatments (Mean 8.3 ± 1.61 SE) (Paired t-test, t = 1.130, P = 0.310). Similarly, the swimming velocity did not differ among individuals between control (Mean 13.1 ± 2.52 SE cm s^{-1}) and HEWAF exposure treatments (Mean 12.7 ± 1.71 SE cm s^{-1}) (Paired t-test, t = 0.504, P = 0.635).

Electric charge distribution around the dipole was also measured in uncontaminated seawater and 100% HEWAF oil solution along the dipole axis at various distances from the dipole center (1, 2, 3, 4, 5, 10, 15, 20, 25 cm). The voltage did not differ significantly between uncontaminated and oil-laden seawater at any distance (t-test; 1 cm P = 0.57, 2 cm P = 0.44, 3 cm P = 0.63, 4 cm P = 0.47, 5 cm P = 0.97, 10 cm P = 0.52, 15 cm P = 0.46, 20 cm P = 0.91, 25 cm P = 0.37). A power curve was fitted to the mean voltage at each distance for oil ($y = 0.7641x^{-0.685}$, $R^2 = 0.891$) and uncontaminated seawater ($y = 0.7656x^{-0.7}$, $R^2 = 0.908$) treatments (Fig. 4). Neither the base nor the exponent differed significantly between treatments (t-test; t = 0.010, P = 0.99 for the base and t = -0.669, P = 0.54 for the exponent).

4. Discussion

This study is the first to quantify the behavioral responses of an elasmobranch to a dipole electric stimulus before and after crude oil exposure. This behavioral assay is used as a proxy to assess the impact of crude oil on electrosensory function. The electrosensory pores on the surface of the skin are directly exposed to the external environment, making them susceptible to environmental pollutants such as crude oil. We found that the distance at which stingrays initiated an orientation to an electric field was reduced by ~2 cm after just five hours of crude oil exposure. This suggests that the stingrays failed to initiate a response to the electric field until they were much closer to the source. A 2 cm reduction may be critical to a small stingray species like *H. sabinus* which has a maximum disc width of approximately 26 cm (Last et al., 2016). Reducing the search field on either side of the animal by 2 cm reduces the overall search field by over 15% of their body width. This reduction in detection distance may ultimately hinder their ability to detect predators, prey items, or mates.

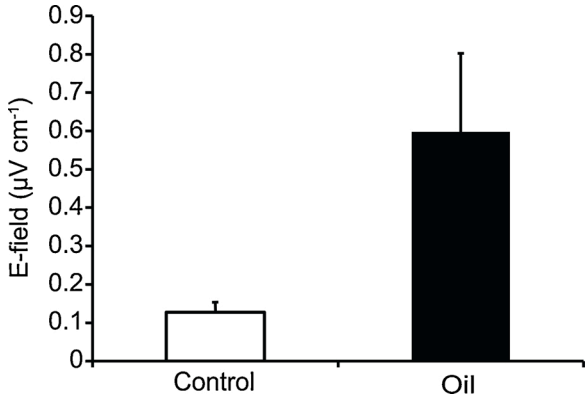

Fig. 3. The mean electric field intensity (+SE) that was required to elicit a bite response in control and 5 h HEWAF-exposure treatments. HEWAF-exposed stingrays initiated a response to an electric field at a significantly greater stimulus intensity.

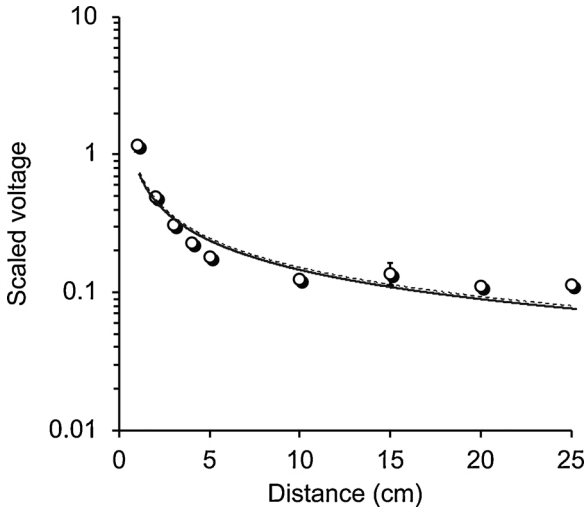

Fig. 4. Voltage measured along the dipole axis at various distances from the dipole center for uncontaminated seawater (open circles) and 100% HEWAF (filled circles). The measured voltage did not differ significantly between the two treatments indicating that the presence of the hydrocarbons within the oil did not change appreciably the electrical properties of the seawater. Values are represented as relative voltage with the mean value set to 1 at 1 cm from the dipole center.

Additionally, detection distance is inversely proportional to electric field intensity. A shorter orientation distance means that the stingrays initiated a behavioral response to a greater electric field intensity. Under crude oil conditions the stingrays initiated their response from a distance closer to the dipole, and hence to a larger magnitude electric field. This suggests that oil exposed animals have an impaired ability to detect potential prey items. These data support the hypothesis that crude oil negatively impacts the ability of *H. sabinus* to respond to bioelectric fields after only a brief exposure.

Electroreceptive capabilities were also tested after 48 hours of exposure to HEWAF. However, after 48 hours of exposure, stingrays generally exhibited reduced activity and failed to feed. Only one stingray that was tested after 48 hours oriented and bit at an active dipole. The orientation distance for that individual was less than its orientation distance in uncontaminated seawater, and similar to its orientation distance after five hours of HEWAF exposure. Because so few bites at the dipole were recorded, the data could not be tested statistically. The lack of data after 48 hours could be explained by stressors that decrease the motivation to feed, or stingrays may simply be incapable of detecting electric fields after prolonged exposure to crude oil as a result of physiological impairment to the ampullae of Lorenzini. Teleosts exposed to various chemical pollutants exhibit a similar decrease in feeding activity, which often develops into a complete discontinuation of feeding when they are subjected to a prolonged exposure or to a higher concentration (Lett et al., 1976; Farmer et al., 1979; Bryan et al., 1995; Kasumyan and Morsi, 1998). Feeding motivation was assessed by quantifying the swimming velocity during searching and by the number of orientations and bites to an active dipole in control and HEWAF treatments. The swimming velocity and the number of orientations and bites to an active dipole did not differ between control and HEWAF treatments after five hours of oil exposure. This suggests that the

stingrays were equally motivated to forage and bite at the dipole even after five hours of HEWAF exposure. Despite still being motivated to feed, the stingrays already exhibited reduced electrosensory sensitivity. Thus, the reduced orientation distance seen after five hours of HEWAF exposure could be attributed to impaired function of the electrosensory system.

No previous studies have examined the effects of environmental pollutants on the electrosensory system, so we can only speculate as to the mechanism of crude oil's effect. One possibility is that the oil impacted the conductivity of the seawater. Hydrocarbons can act as electrical insulators and create a high impedance pathway. The voltage around a dipole was empirically measured and the charge distribution of the electric field did not differ between uncontaminated seawater and 100% HEWAF crude oil solution. Since the 100% HEWAF had a much greater concentration of hydrocarbons than the oil exposure treatment used in the behavioral assay, yet did not change the electric field properties, it is unlikely that the more dilute oil exposure treatment could be responsible for the decreased orientation distance. In addition, the electric field was measured within 2 h of the HEWAF being prepared. This is important because the aromatic hydrocarbons in the HEWAF will evaporate over time and reduce the concentration within the solution. By measuring the electric field in the seawater as soon as possible when the hydrocarbon concentration is greater, any effects that the hydrocarbons impose would be even greater than we would expect during the behavioral assays which took place after 5 hours. Since the conductivity of the uncontaminated and HEWAF treated seawater did not differ after a maximum of 2 hours, when hydrocarbon concentrations were high, the longer duration exposure during the behavioral assay would be to an even more dilute concentration. Therefore, since the physical properties of the seawater do not appear to account for the reduced orientation distance, it is probable that the effects seen in stingrays are possibly due to the crude oil physiologically interacting with the animal. One possibility is that the crude oil might adhere to the skin and form a high impedance electrical barrier over the pores that lead to the ampullae of Lorenzini. This insulative layer could hinder or prevent the stimulus from reaching the sensory cells. However, since the electrical conductivity did not differ between the uncontaminated and HEWAF seawater, it seems unlikely that the petroleum products in the oily seawater would be sufficient to form an electrical barrier. A high impedance barrier might form only if the hydrocarbons selectively bind to or react with the conductive glycoprotein gel in the ampullary canals, causing the gel to be less electrically conductive. A recent study found that keratan sulfate in the glycoprotein gel within the ampullary canals is responsible for contributing to its high proton conductivity (Josberger et al., 2016). Various constituents of crude oil might interact with keratan sulfate, or other constituents of the glycoprotein gel, and change its electro-chemical properties. A less conductive glycoprotein gel would impede electrical transmission to the sensory cells found in the ampullae, thus reducing the ability to detect a bioelectric field. Given the fact that the electric fields to which the stingrays respond are very weak, even a small change in the gel conductivity could impact responsiveness. Finally, crude oil elements might be transported down the canal through the glycoprotein gel and cause cellular damage to the sensory cells within the ampullae. This option is unlikely since the effects were seen after only five hours of exposure. Regardless of the mechanism, the end result is that even short-term oil exposure significantly reduced behavioral response to prey-simulating electric stimuli.

An alternative to explain the greater stimulus required to initiate a bite during the HEWAF exposure treatment might be that the stingrays learned that there was no reward for biting and hence did not exhibit the same vigorous reaction when re-tested. Because the stingrays failed to receive a food reward immediately after they bit at the electrodes, they might have been less motivated to bite when they were exposed to the electrodes subsequently, during the HEWAF exposure trials. Elasmobranchs have exhibited the ability to orient and retain spatial memory, learn with social context, recognize and avoid objects, and retain memory (Schluessel, 2015; Newton and Kajiura, 2020). Recent work demonstrated that the yellow stingray (*Urobatis jamaicensis)* learned to discriminate magnetic stimuli in as few as 10 sessions of training consisting of 40 individual trials (Newton and Kajiura, 2017). Because stingrays in the current project were tested twice, and the oil treatment was always tested after the uncontaminated seawater treatment, the possibility exists that the stingrays learned to not bite at the electrodes. However, this would require that the animals learn to make an association between a prey-simulating electric stimulus and a lack of reward after only one experimental session consisting of a maximum of 12 individual trials. This seems unlikely given that other stingray species required multiple training sessions to establish a learned behavior. Therefore, it is most probable that the reduced response is due to a physiological impairment of their electrosensory system rather than a learned behavior. To further support this claim, the swimming velocity during searching and the number of bites at the target dipoles was not decreased during the HEWAF exposure treatments which suggests that the animals were similarly motivated for both control and HEWAF exposure treatments.

Based upon the results of this study, electroreceptive function appears to be impaired after exposure to crude oil, and it would be interesting to determine how long it takes for the animals to recover to pre-exposure capability after being returned to uncontaminated seawater. Future studies should test animals prior to oil exposure, while the animals are in oily water, and after they have been returned to uncontaminated seawater again. This would clarify if stingrays have the capability to recuperate normal electroreceptive function after an oil spill and could shed some light on the mechanism responsible for the impairment. For example, if the stingrays were able to quickly regain function, it would suggest that the impairment is not due to sensory cell damage.

The concentration of oil to which the stingrays were exposed in this study was an average of oil concentrations empirically measured along the Gulf of Mexico coastline following the Deepwater Horizon disaster (Sammarco et al., 2013; Incardona et al., 2014). Given that this average concentration induced a significant impairment in electric stimuli detection, higher concentrations are likely to have an even greater impact over a shorter exposure duration. The polycyclic aromatic hydrocarbons (PAHs) that are a major constituent of the Deepwater Horizon crude oil are volatile and will evaporate over time and reduce the concentration of contaminant within the seawater (Havenga and Rohwer, 2002; Plata et al., 2008; Liu et al., 2012). However, PAHs and other toxic constituents can still remain within the substrate and be released slowly over time thus providing a protracted exposure period (Liu et al., 2012). These substratum components have the potential to impact benthically associated species, such as the stingrays which bury into the sediment. It has been documented that various fish species reduce swimming activity in oily water and avoid oil contaminated sediments (Amiard-Triquet et al., 2012; Martin, 2017) so the impact may be mitigated by the behavior of the animals.

The impact of crude oil has been largely overlooked in the elasmobranch fishes, despite their importance as meso- and upper-trophic level marine predators. Therefore, how sensory function is impaired by anthropogenic stressors in this group of fishes is an area ripe for fruitful research.

Declaration of Competing Interest

The authors report no declarations of interest.

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