Electroreception in the euryhaline stingray, *Dasyatis sabina*

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SUMMARY

This study quantified the electrosensitivity of a euryhaline elasmobranch, the Atlantic stingray (*Dasyatis sabina*) across a range of salinities. Specimens from a permanent freshwater (FW) population in the St Johns River system, FL, USA, were compared with stingrays from the tidally dynamic Indian River Lagoon in east Florida, USA. Behavioral responses of stingrays to prey-simulating electric stimuli were quantified in FW (0 p.p.t., $\rho=2026\,\Omega\,cm$), brackish (15 p.p.t., $\rho=41\,\Omega\,cm$) and full strength seawater (35 p.p.t., $\rho=19\,\Omega\,cm$). This study demonstrated that the electrosensitivity of *D. sabina* is significantly reduced in FW. In order to elicit a feeding response, stingrays tested in FW required an electric field 200–300× greater than stingrays tested in brackish and saltwater (median FW treatments=1.4 μV cm^{-1}, median brackish–saltwater treatments=6 nV cm^{-1}), and the maximum orientation distance was reduced by 35.2%, from 44.0 cm in the brackish and saltwater treatments to 28.5 cm in FW. The St Johns River stingrays did not demonstrate an enhanced electrosensitivity in FW, nor did they exhibit reduced sensitivity when introduced to higher salinities. Stingrays from both populations responded similarly to the prey-simulating stimulus when tested at similar salinities, regardless of their native environment. The reduction in electrosensitivity and detection range in FW is attributed to both an environmental factor (electrical resistivity of the water) and the physiological function of the ampullary canals. The plasticity of this sensory system to function across such a wide environmental range demonstrates its adaptive significance.

INTRODUCTION

Sensory systems are optimized to function in the medium through which the stimuli are transmitted. For most sensors, the properties of the medium are relatively constant. For example, air and water density do not vary much within a biologically relevant range, which allows hearing or lateral line senses to function similarly in any environment an organism will encounter during its lifetime. An exception is the electrical properties of water, which vary dramatically with salinity, resulting in a conductivity difference of more than two orders of magnitude between seawater and freshwater (FW). For euryhaline species that regularly encounter dramatically different environmental conditions throughout their life, an electrosensory system would need to demonstrate corresponding plasticity to function across such a wide range of conductivities.

All elasmobranch fishes (sharks, skates and rays) possess an extremely sensitive electrosensory system that enables them to detect weak extrinsic electric fields in their environment. This sensory capability has been demonstrated to function in the detection and localization of weak bioelectric fields of less than 0.5 nV cm^{-1} generated by prey (Kalmijn, 1971; Tricas, 1982; Haine et al., 2001; Kajiura and Holland, 2002) and conspecifics (Tricas et al., 1995). It has also been shown to detect the relatively low-frequency electric fields produced by predators (Sisneros et al., 1998), and is theorized to aid in geomagnetic orientation and navigation (Kalmijn, 1974; Paulin, 1995).

The elasmobranch electrosensory system consists of hundreds to thousands of bulb-like electroreceptive organs known as the ampullae of Lorenzini. These ampullae are grouped into three to five subdermal clusters that are distributed over the head of galeoids and the head and body of batoids (Chu and Wen, 1979; Zakon, 1988). A single ampulla consists of multiple alveolar sacs continuous

with a narrow canal that terminates in a pore on the skin surface (Waltman, 1966). The canal wall is comprised of cells that are bound by tight junctions that form a higher impedance electrical barrier than the glycoprotein gel within the canal (Murray and Potts, 1961; Waltman, 1966; Brown et al., 2002). The lumen of the ampulla is lined by a single-layer epithelium that is comprised of sensory and support cells (Waltman, 1966; Zakon, 1988; New and Tricas, 2001).

The elasmobranch electrosensory system evolved in a highly conductive seawater environment. However, there are several elasmobranch species that have subsequently transitioned to a FW environment. The high impedance FW environment presents a challenge to their electrosensory system. The obligate FW South American stingrays (Potamotrygonidae) possess a much thicker skin than their marine ancestors, which enables the animal to maintain an internal ionic concentration that is greater than that of the surrounding FW (New and Tricas, 2001). The thicker skin also forms a high impedance barrier that results in a large voltage differential across the skin (Kalmijn, 1974; Raschi and Mackanos, 1989; New and Tricas, 2001). The ampullae are significantly smaller (microampullae), are individually distributed in the dermis rather than in subdermal clusters, and the canals are much shorter (Raschi and Mackanos, 1989; New and Tricas, 2001). Thus, the subdermal location of the microampullae facilitates detection of these transcutaneous voltage changes (Kalmijn, 1974; Zakon, 1988; New and Tricas, 2001).

Whereas the electrosensory systems of FW elasmobranchs have evolved to function in a high impedance environment, the electroreceptors of euryhaline elasmobranchs remain morphologically undifferentiated from exclusively marine species (Whitehead, 2002). Nonetheless, euryhaline species retain electroreceptive capabilities in FW, as demonstrated in the bull shark

(*Carcharhinus leucas*) (Whitehead, 2002). How the function of the elasmobranch electric sense is affected when euryhaline species enter FW remains unknown.

The effect of decreasing salinity on the electrosensory system is of particular interest because many euryhaline elasmobranchs are widely distributed across a range of salinities. These include several species of stingrays commonly found in FW tributaries and upstream of coastal areas, including *Dasyatis guttata*, *Dasyatis garouaensis*, *Dasyatis bennetti* and *Dasyatis sephen* (Thorson et al., 1983; Thorson and Watson, 1975; Taniuchi, 1979). Larger elasmobranchs, such as the bull shark (*C. leucas*) and the largetooth sawfish (*Pristis perotteti*) also frequent FW systems but they continue to utilize the marine environment for some critical life stages (Springer, 1963; Thorson et al., 1966; Bass et al., 1973; Thorson, 1974; Thorson, 1976; Jensen, 1976; Taniuchi, 1979; Snelson et al., 1984).

The goal of this study was to determine the electroreceptive capabilities of a euryhaline elasmobranch throughout the range of salinities and hence conductivities that it would encounter in its natural environment. The organism selected for this study was the Atlantic stingray (*Dasyatis sabina*). This species is locally abundant throughout the primarily brackish Indian River Lagoon (IRL) system in east Florida, USA, where it is found over shallow open sand and silt bottoms, associated with sea grass beds and spoil islands (Snelson and Williams, 1981; Snelson et al., 1988). It has a broad diet of benthic fauna that primarily consists of amphipods and mysids (Cook, 1994). The presence of a permanent FW population in the nearby St Johns River (SJR), FL, USA, provided the opportunity to compare electrosensory capabilities of populations of the same species that inhabit electrically dissimilar environments. The SJR population completes its full life cycle in FW, with no significant differences in size of maturity and reproductive success compared with marine populations in the nearby IRL and the northeast Gulf of Mexico (Johnson and Snelson, 1996). To our knowledge, *D. sabina* is the only marine elasmobranch that has established a permanently FW population. Johnson and Snelson (Johnson and Snelson, 1996) hypothesized that the population may have undergone adaptations to its FW environment, although there are no known morphological differences between the SJR population and any other marine *D. sabina* populations. A comparison of the two populations may provide insight into possible evolutionary adaptations and plasticity of the electrosensory system in this species. Therefore, the objectives of this study were to behaviorally determine the electrosensitivity of the Atlantic stingray to prey-simulating electric fields in marine (35 p.p.t.), brackish (15 p.p.t.) and FW (0 p.p.t.) treatments, and to then compare the electrosensitivities of stingrays from the IRL population with that of the SJR population to determine if permanent exposure to FW has affected the electric sense in this species.

MATERIALS AND METHODS
Study area
The IRL system is a dynamic estuary located along the east coast of Florida, USA (Fig. 1). The estuary undergoes wide-ranging salinity fluctuations throughout the year, with seasonal changes ranging from full-strength seawater in the winter/spring dry season to FW in the late-summer/autumn wet season. In the southern half of the IRL, daily salinity fluctuations result from tidal flushing through four inlets connected to the Atlantic Ocean, mixed with FW inflows from rivers, creeks and canals draining from the interior of the Florida peninsula. Atlantic stingrays (*Dasyatis sabina* Leseur 1824; Dasyatidae) in the IRL are found in all salinities and are tolerant of moderate daily fluctuations (Snelson

and Williams, 1981; Snelson et al., 1988) (D.W.M., personal observation).

The SJR system originates along the east coast of Central Florida, USA as a series of interconnected FW lakes that merge to form a northward flowing river, eventually emptying into the Atlantic Ocean through Jacksonville, FL, USA. Lake Harney (28 deg.45′N, 81 deg.03′W) (Fig. 1) is a 3211 ha FW lake in this system, located approximately 300 km south of the mouth of the SJR.

Stingray collection and maintenance
Stingrays were collected using equipment that avoided any damage to the electroreceptors (i.e. hook wounds near the mouth). In the IRL, stingrays were collected between August 2005 and January 2007 using a 183.5 m center-bag seine. In Lake Harney, stingrays were collected between June 2006 and August 2006 by electrofishing from an airboat. Upon capture, each stingray was sexed, measured and placed in a live well. Only sexually mature stingrays (>22 cm disc width) were retained for this study to account for previously described ontogenetic changes in response properties of the Atlantic stingray's electrosense (Snelson et al., 1988; Sisneros and Tricas, 2002).

Stingrays caught in the IRL at salinities less than 7 p.p.t. were assigned to the FW IR0 treatment (0 p.p.t., $\rho=2026\,\Omega\,cm$), those captured at salinities between 7.1 and 24.9 p.p.t. were assigned to the brackish-water IR15 treatment (15 p.p.t., $\rho=41\,\Omega\,cm$) and stingrays captured in salinities greater than 25 p.p.t. were assigned to the saltwater IR35 treatment (35 p.p.t., $\rho=19\,\Omega\,cm$). A major drought in 2006 resulted in higher salinities throughout the IRL, which precluded collection of stingrays for the IR0 FW treatment. As a result, seven brackish water stingrays were acclimated to FW by decreasing salinity levels by 2 p.p.t. every 24 h, and were then assigned to the IR0 treatment. Because the SJR was always fresh, all SJR stingrays were assigned to the FW SJ0 treatment.

All stingrays were maintained in identical $122\times244\times50\,cm$ fiberglass holding tanks at either the Florida Atlantic University (FAU) Marine Science facility or the Boca Raton campus, FL, USA. The IR35 stingrays were held at the FAU Marine Science facility in marine flow-through aquaria at 23.0–26.8°C. The IR0, IR15 and SJ0 stingrays were held at the FAU Boca Raton campus in closed-circuit flow tanks at 21.3–22.0°C. All rays were fed to satiation once daily on a diet of thawed grass shrimp.

Experimental apparatus
The experimental tank was identical to the holding tank and connected to the same water flow and filtration system. An opaque $122\times213\,cm$ acrylic plate lay on the bottom of the experimental tank. An identical plate was also placed in the bottom of the holding tank to replicate the environment of the experimental tank. Four electric dipoles were equally spaced 40 cm apart from one another in four quadrants of a $1\times1\,m$ square near one end of the acrylic plate (Fig. 2). Each dipole consisted of a pair of holes drilled into the plate. The gap distance between the centers of the dipole openings were 1 cm, which simulated a small prey item. Under each opening, a water-filled polyethylene tube was mounted flush to the underside of the acrylic plate to act as a salt bridge. The polyethylene tubing was 50 cm in length and connected to gold-plated stainless steel pins terminating from a shielded 18 AWG SO underwater cable (Teledyne Impulse, San Diego, CA, USA). Each dipole's pair of cables was connected to an electric stimulator based on Kajiura and Holland's (Kajiura and Holland, 2002) design that would produce a prey-simulating dipole electric field. The electric field produced

by the dipole was maintained at a constant intensity by continuous adjustment of the applied current from 6.9 to 8.3 μA. Current was randomly delivered to one of the four electric dipoles and was monitored with a multimeter to be within 0.1 μA of the target intensity. A digital video camera mounted on a tripod was positioned 1 m above the surface of the water over the center of the electrode array to record each trial.

Experimental protocol

Trials were conducted with individual stingrays after they had been acclimated in their holding tanks to the test salinities of either 0 p.p.t. (ρ=2026 Ω cm), 15 p.p.t. (ρ=41 Ω cm) or 35 p.p.t. (ρ=19 Ω cm) and had been feeding for a minimum of seven days. Prior to commencement of experimental trials, stingrays were fasted for a minimum of 48 h. One stingray was moved from the holding tank to the experimental tank and allowed to acclimate for a minimum of 10 min. The stingray was aroused to feed by placing one piece of thawed shrimp in the experimental tank to elicit a prey-searching behavior, determined by increased swimming velocity and turning frequency. Once the stingray ingested the shrimp and began to search for more food, video recording commenced and the stimulator was switched on to deliver current to one of the four dipoles. The electric field was maintained until the stingray was observed to bite at the active dipole, resulting in a positive response, at which time the stimulator was immediately switched off. The stimulator was reactivated when the stingray was at least 80 cm away from the next randomly selected dipole, which represented twice the distance of the maximum observed response. This procedure was repeated until a positive response was observed at each dipole or until the stingray no longer showed interest (defined by either no longer biting at the active dipole or by not exhibiting a prey-searching behavior), ending the trial. The stingray was then fed to satiation and returned to its holding tank. Stingrays that did not exhibit a prey-searching behavior or motivation to feed were subjected to a second trial under the same experimental conditions.

The IRL stingrays were tested only at the same salinity in which they were captured (i.e. 0, 15, 35 p.p.t.). The SJR stingrays were tested first in FW (0 p.p.t.) then slowly acclimated to 15 p.p.t., increasing the tank salinity by 2 p.p.t. every 24 h. Once acclimated to brackish water and feeding normally for a minimum of one week, trials were conducted and the results constituted the SJ15 treatment. The stingrays were then acclimated to 35 p.p.t. following the same protocol and tested again in saltwater (SJ35 treatment).

Data analysis

Video clips of successful responses were extracted from the source tapes and edited using the software Final Cut Pro (Apple, Cupertino, CA, USA). All video clips were renumbered prior to analysis to
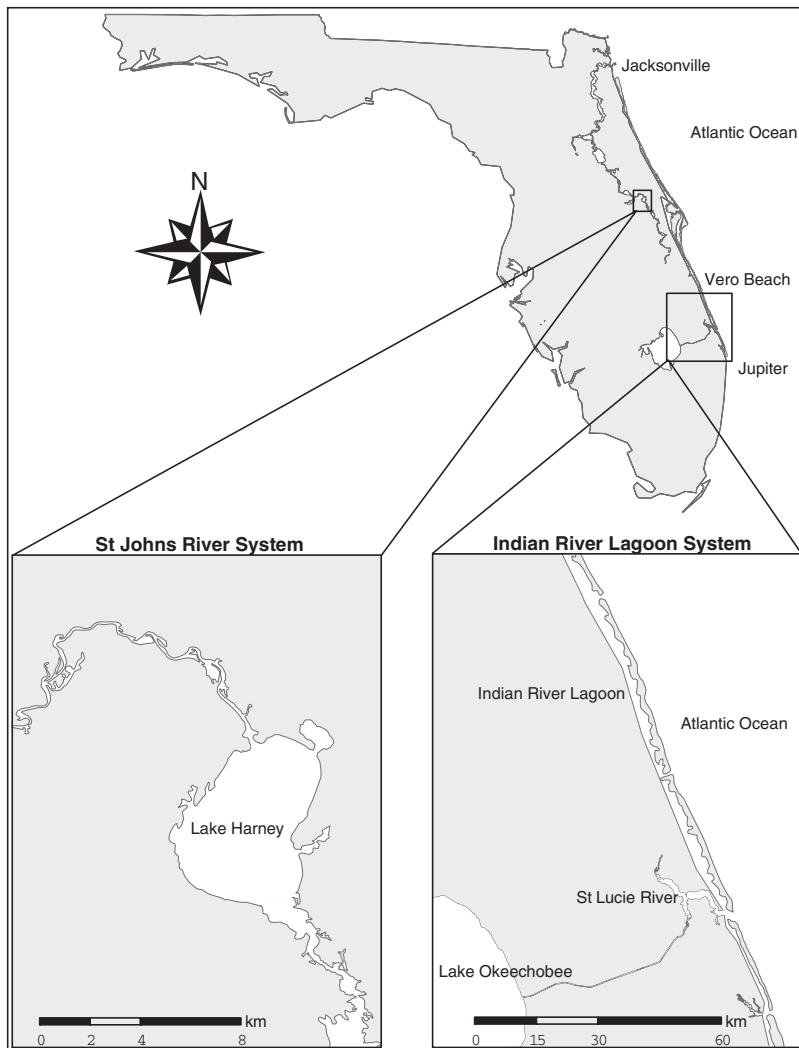

Fig. 1. Map of the two sample areas: Lake Harney in the St Johns River system, located approximately 300 km upstream from its saltwater outlet in Jacksonville, FL, USA and the Indian River Lagoon (IRL) system along the east coast of FL, USA. For this study, stingrays were only collected from the southern half of the IRL, ranging from Vero Beach, FL, USA (27 deg.38′N, 80 deg.22′W) as the northern boundary to Jupiter, FL, USA (26 deg.58′N, 80 deg.05′W) as the southern boundary, and including the St Lucie River estuary to the west (north fork 27 deg.14.50′N, 80 deg.19.00′W and south fork 27 deg.10.00′N, 80 deg.15.25′W). The C-44 canal in the southwest corner of the St Lucie River connects Lake Okeechobee to the IRL and is a major source of freshwater inflow into the study area.
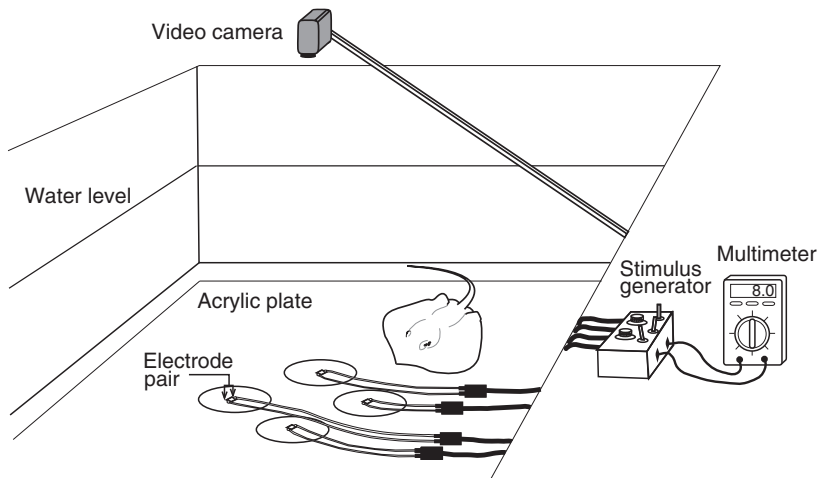
conceal the treatment and test salinity, thereby enabling the analysis to be conducted in a single-blind fashion to prevent bias. From the resultant video clips the frame in which the stingray initiated an orientation to the dipole was extracted. The point at which the orientation was initiated was determined by observing one or a combination of three behaviors: a freeze response, where the stingray rapidly ceased to undulate its pectoral fins, a flaring out of the pelvic fins to slow its forward motion or a sudden change in swimming trajectory (Kajiura and Holland, 2002). The response frame was contrast adjusted and deinterlaced using the software Adobe Photoshop (Adobe Systems, Mountain View, CA, USA) to make the stingray's spiracle more visible. From this frame, the stingray's distance from the center of the dipole to the point at which the orientation was initiated (orientation distance) and the angle of the orientation point with respect to the dipole axis (orientation angle) were quantified using the software ImageJ (NIH, Bethesda, MD, USA). Orientation distance was measured from the center of the dipole to the posterior margin of the closest spiracle, which closely approximates the position of the hyoid ampullary cluster (see supplementary movie). The responses for each stingray were reviewed by a second analyst in a single-blind fashion to verify the initiation point for the orientation to the dipole. The results of both analysts were in agreement within one frame of the orientation point for all responses.

The orientation distance and angle were incorporated into the ideal dipole field equation (Griffiths, 1989) to determine the electric field at the point where the stingray first oriented towards the simulated prey (Kalmijn, 1982). The ideal dipole equation states:

$$E = \cos\theta \, \frac{\rho \, I d}{\pi \, r^3} \,, \qquad (1)$$

where E is the electric field ($\mu V \, cm^{-1}$), ρ is the resistivity of the water (the experimental factor, $\Omega \, cm$), I is the applied current (6.9–8.3 μA), d is the gap distance between the centers of the dipole openings (1 cm), r is the measured orientation distance (cm) and θ is the measured orientation angle with respect to the dipole axis (deg.). The orientation angle is included in the equation to account for the cosine dependency of the electric field (Kalmijn, 1982).

The weakest electric field that elicited a positive response for each stingray was defined as its best response and used for statistical analysis. A natural log transformation was applied to the derived electric field values to meet the assumptions of normality and homoscedasticity (Ramsey and Schafer, 2002). A one-way analysis of covariance (ANCOVA) was employed to test for differences in

the minimum detected electric field among the treatments, with disc width included as the covariate (Dowdy and Wearden, 1991). All statistical analyses were conducted using SAS version 8.02 (Cary, NC, USA).

To better illustrate the functional detection range of the stingrays in the different environments, a two-dimensional detection area around the dipole was graphically depicted. The detection range was plotted on a 90 deg. polar plot by calculating r, derived from median E, at all angles from 0–90 deg. (θ) for each environment. This graphical representation of the detection area around the dipole exemplifies the cosine dependency of the electric field. This approach was also used to calculate the maximum detection range using the single weakest electric field for each environment.

RESULTS

Fifty-one Atlantic stingrays were collected and tested for this study. Ten were collected in FW from Lake Harney in the SJR system. Within the IRL system, four were collected in FW, 18 in brackish water and 11 in saltwater. One male stingray (IR0-04) was retained even though its disc width was less than 22 cm (i.e. 21.8 cm) because its claspers were elongated and fully calcified. This indicated that the stingray was sexually mature therefore meeting the study requirements of testing only sexually mature adults (Snelson et al., 1988).

The SJR stingrays were successfully acclimated from 0 p.p.t. to 15 p.p.t. but experienced high mortality (60%) immediately after the second acclimation period from 15 to 35 p.p.t. Logistical constraints precluded collecting additional stingrays from Lake Harney, resulting in the exclusion of the SJ35 treatment from the analysis.

Electric field detection threshold

Of the 51 stingrays tested, seven were excluded from the analysis due to lack of motivation to feed during trials. All observed responses entailed either a straight trajectory into the active dipole followed by a freeze and pelvic fin flare-out or a single turn in swimming trajectory. The size and electrosensitivity of the remaining experimental rays is summarized for each treatment in Table 1. Each stingray's best response (i.e. lowest electric field) is shown in Fig. 3. Best responses in all treatments showed the orientation distance from the dipole decreased as the stingray approached from a higher angle to the dipole axis (Fig. 4). The strength of the electric field differed significantly among the five treatments (ANCOVA; $F_{5,39}$=70.79, P<0.0001, R^2=0.90), and the disc width covariate did not

Table 1. Stingray disc width and observed electric field that resulted in initiation of a feeding response by treatment

	N	Mean disc width ±s.e.m. (mm)	Disc width range (mm)	Mean treatment resistivity ±s.e.m. (Ω cm)	Median electric field ±s.e.m. (μV cm^{-1})	Electric field range (μV cm^{-1})
Treatment						
IR0	10	252.1±8.4	218–308	2087±151.6	1.2±0.5	0.2–5.5
IR15	9	247.6±3.7	230–265	41±0.4	0.01±0.002	0.002–0.02
IR35	8	252.9±4.2	232–265	19±0.2	0.005±0.007	0.0006–0.06
SJ0	10	283.8±8.2	244–339	1965±49.7	1.5±0.5	0.3–5.3
SJ15	7	282.9±5.6	270–312	40±0.02	0.003±0.0007	0.002–0.007
Combined treatments						
FW	20	268.0±6.8	218–339	2026±78.9	1.4±0.3	0.2–5.5
BSW	24	259.6±4.0	230–312	34±2.1	0.005±0.002	0.0006–0.06

Treatment classifications (IR0, IR15, IR35, SJ0, SJ15) are created by combining the stingray capture location, the Indian River Lagoon (IR) or St Johns River (SJ), with the salinity at which they were tested, freshwater (0 p.p.t.), brackish water (15 p.p.t.) or full-strength seawater (35 p.p.t.). FW, freshwater; BSW, brackish–saltwater.

significantly affect the model ($P=0.09$). A Tukey–Kramer *post hoc* test revealed that neither the two FW treatments (IR0 *vs* SJ0, $P=0.99$), nor the saltwater *vs* the brackish treatments (IR15 *vs* IR35, $P=0.60$; IR15 *vs* SJ15, $P=0.19$; IR35 *vs* SJ15, $P=0.84$) differed statistically. However, the median electric field in both FW treatments was significantly greater than each of the brackish–saltwater (BSW) treatments ($P=<0.0001$). The median electric fields in the two FW treatments (IR0=1.2 μV cm^{-1}, SJ0=1.5 μV cm^{-1}) were greater than the median electric fields for the saltwater (IR35=0.005 μV cm^{-1}) and two brackish water treatments (SJ15=0.003 μV cm^{-1}, IR15=0.01 μV cm^{-1}). The weakest electric field detected was 0.0006 μV cm^{-1} by an IR35 stingray whereas the weakest electric field in either FW treatment was 0.2 μV cm^{-1} by an IR0 stingray. These results justified pooling the treatments into two groups: a FW and a BSW treatment group.

Prey detection range

Detection ranges were derived for the two pooled environmental groups, rather than for all five treatments. The point at which the stingray initiated its orientation to the dipole was plotted using polar coordinates for each successful response (Fig. 5A). The median electric fields for the BSW (0.006 μV cm^{-1}) and FW (1.4 μV cm^{-1}) groups were derived from the best responses for each stingray in their respective treatment. In the plane of the dipole axis (0 deg.),

the derived median detection distance was 25.03 cm in BSW and 15.33 cm in FW (Fig. 5B). The maximum detection distance in the plane of the dipole axis (0 deg.) was also calculated using minimum electric field value for each group (BSW=0.6 nV cm^{-1}, FW=0.2 μV cm^{-1}). The derived maximum detection distance was 44.03 cm in BSW and 28.52 cm in FW (Fig. 5B). These values slightly exceeded the maximum observed detection distance of 38.8 cm at 46.5 deg. in BSW and 24.47 cm at 41.1 deg. in FW.

DISCUSSION

The elasmobranch fishes are renowned for their remarkable sensitivity to even very weak electric fields. As most elasmobranchs inhabit a marine environment, most studies of their electrosensory capabilities have been conducted in seawater, which possesses a low electrical impedance. However, the electrical properties of the water they inhabit dramatically impact their electrosensory capabilities. This study is the first to quantify the differences in electrosensitivity of a euryhaline elasmobranch that is regularly exposed to an electrically dynamic environment: one that ranges from a relatively low impedance marine estuary to a relatively high impedance FW system.
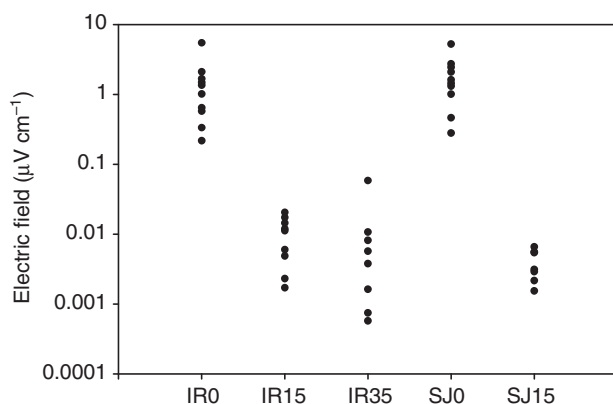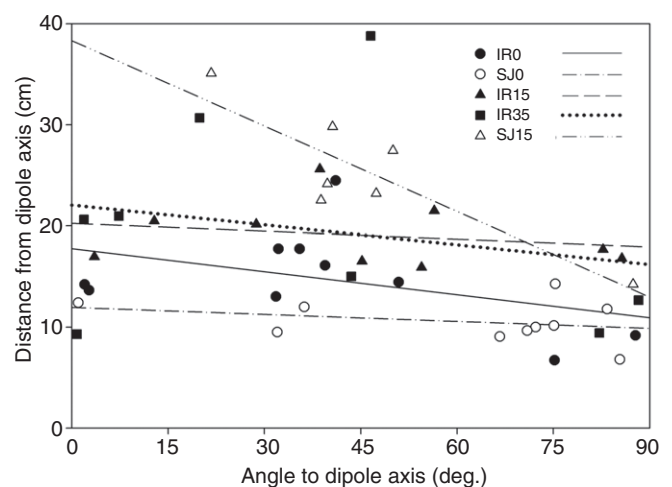
Fig. 3. Electric field values for each stingray's best response to the prey-simulating dipole. Treatment classifications (IR0, IR15, IR35, SJ0, SJ15) are created by combining the stingray capture location, the Indian River Lagoon (IR) or St Johns River (SJ), with the salinity at which they were tested, freshwater (0 p.p.t.), brackish water (15 p.p.t.) or full-strength seawater (35 p.p.t.).

Fig. 4. Scatter plot of orientation distance *vs* angle to the dipole axis for the best response of each treatment. For all treatments, there is a slight but consistent trend for decreasing orientation distance at higher angles. Treatment classifications (IR0, IR15, IR35, SJ0, SJ15) are created by combining the stingray capture location, the Indian River Lagoon (IR) or St Johns River (SJ), with the salinity at which they were tested, freshwater (0 p.p.t.), brackish water (15 p.p.t.) or full-strength seawater (35 p.p.t.).
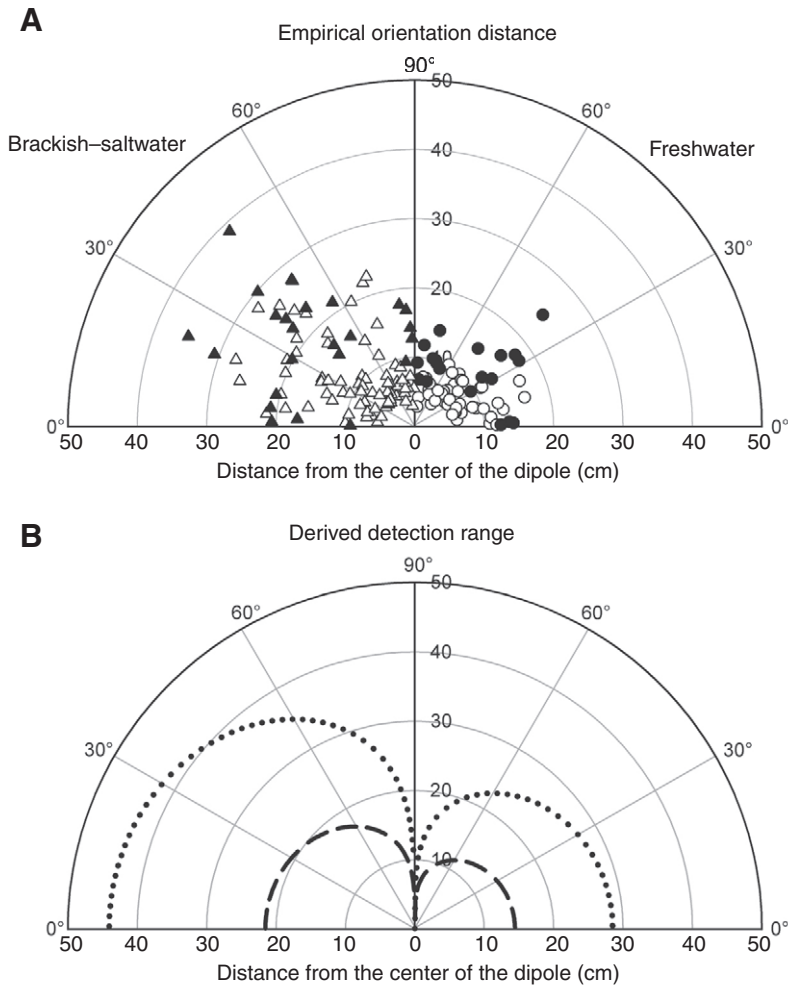
A

Empirical orientation distance

B

Derived detection range

Fig. 5. Empirical orientation distances and derived detection ranges for brackish–saltwater (BSW) (left side) and freshwater (FW) (right side) groups. The center of the dipole that produced the prey-simulating electric field is located at the intersection of the horizontal and vertical axes. Both axes represent the distance from the dipole origin in cm (horizontal axis = distance in the plane of the dipole axis; vertical axis = distance in the plane normal to the dipole axis). (A) The spatial location of each stingray at which an orientation was initiated prior to a successful feeding response. The responses are depicted within a 90 deg. quadrant for both groups (△ = all response points for BSW group; ▲ = best response points for each stingray in the BSW group; ○ = all response points for FW group; ● = best response points for each stingray in the FW group). (B) The derived detection range as the median detection distance (dashed line) and the maximum detection distance (dotted line). See text for further details.

The Atlantic stingray *D. sabina* is able to detect and orient towards a prey-simulating, weak electric field across a wide range of salinities, including FW. Interestingly, despite the electric field strength being much greater in FW, the sensitivity of the electrosensory system appears to be reduced. This is evidenced by the detection range and electrosensitivity of the stingrays in both FW treatments being significantly less than those tested in the BSW treatments. The derived median and maximum orientation distances of stingrays tested in FW were reduced by 38.8% and 35.2%, respectively, compared with the combined BSW treatments. Differences in electrosensitivity were even more pronounced, as the stingrays tested in FW required a stimulus intensity of 200–300× greater to elicit a behavioral response. Furthermore, this difference was retained regardless of whether the stingrays were captured from the permanent FW population or the marine estuary. This reduced sensitivity is akin to prey items producing a signal that is 200× 'louder' in FW but the stingrays are unable to detect it.

When compared with the IRL stingrays, the FW SJR stingrays did not demonstrate a significantly enhanced electrosensitivity in FW, nor did they exhibit a significantly reduced electrosensitivity in brackish water. In fact, the electrosensitivities did not differ between the two populations in either FW or brackish water. The response of the FW SJR stingrays that were acclimated to brackish water, as well as the IRL stingrays caught in brackish water that were acclimated to FW, were similar to the responses of other stingrays from both populations that were tested at those salinities

without acclimation. This demonstrates the plasticity of the electrosensory system, as individuals from one environment transferred to another were able to function similarly to individuals native to that environment. To minimize the stress of the acclimation process, prevent osmotic shock and maintain normal feeding behavior, the stingrays were slowly acclimated to the test salinity ($\pm$2 p.p.t. day^{-1}) and then held at the test salinity for a minimum of one week. Previous studies subjected stingrays collected from the SJR to faster rates of acclimation up to 32 p.p.t. but the effects of the rapid acclimation on feeding behavior are unknown because the stingrays were food-deprived throughout the one-month trial (Piermarini and Evans, 1998).

The electrosensitivities determined for the BSW stingrays are consistent with previous studies that tested other elasmobranch species in marine environments with a similar experimental apparatus. Small smooth dogfish (*Mustelus canis*) initiated responses to prey-simulating electrical stimuli of <2 nV cm^{-1} from >35.6 cm (Kalmijn, 1982). Juvenile scalloped hammerhead sharks (*Sphyrna lewini*) and sandbar sharks (*Carcharhinus plumbeus*) demonstrated minimum electrosensitivities of 0.4 nV cm^{-1} and 0.5 nV cm^{-1} and maximum detection distances of 30.6 cm and 31.6 cm, respectively (Kajiura and Holland, 2002). Neonatal bonnethead sharks (*Sphyrna tiburo*) responded to electric fields of <1 nV cm^{-1} from a maximum detection distance of 22 cm (Kajiura, 2003). Direct comparison of the results of FW stingrays with the results of other FW elasmobranchs is limited due to a lack of quantified physiological
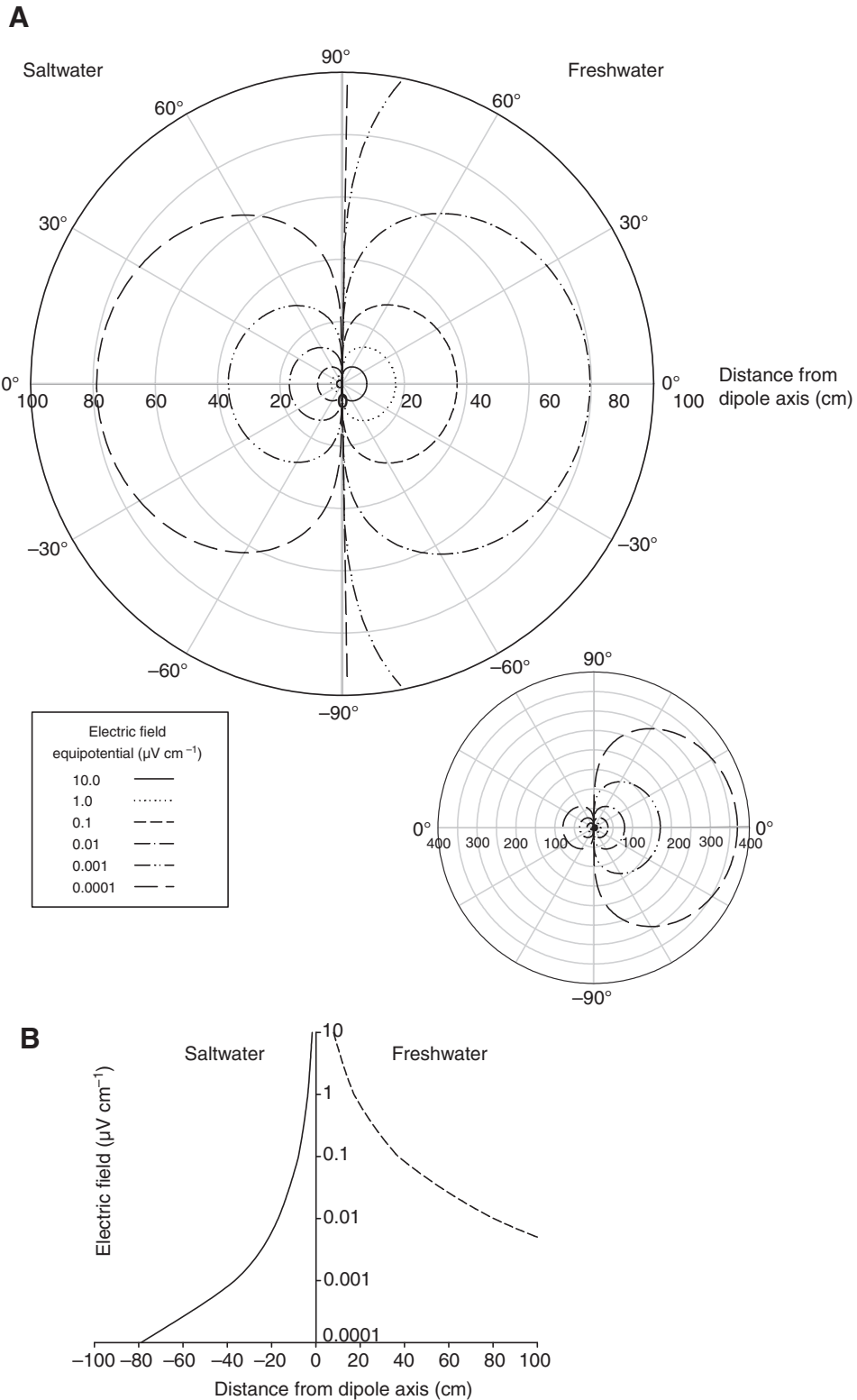
A

B

Fig. 6. The distribution of voltage equipotentials produced by a prey-simulating electric dipole in an electrically conductive marine environment (left) and a resistive freshwater (FW) environment (right). Electric fields were modeled using the same parameters as in this study (i.e. 8 μA applied current, 1 cm dipole opening separation, seawater resistivity of 19 Ω cm and FW resistivity of 2026 Ω cm). In A, each equipotential represents an order of magnitude of the electric field, ranging from 10.0 μV cm^{-1} to 0.001 μV cm^{-1}. The center of the dipole that produced the electric field is located at the intersection of the horizontal and vertical axes. Both axes represent the distance from the dipole origin in cm (horizontal axis = distance in the plane of the dipole axis; vertical axis = distance in the plane normal to the dipole axis). The inset in the lower right corner illustrates the full extent of the electric field in FW. (B) A representative slice through A at 0 deg. to the dipole axis. It depicts the rate at which an electric field decreases as distance from the dipole increases in seawater (19 Ω cm – left side) and FW (2026 Ω cm – right side) on a semi-log scale. Note that the electric field decreases much more dramatically with distance in seawater compared with FW.

or behavioral sensitivities of the electrosensory system in previous studies. Szabo et al. (Szabo et al., 1972) reported that the obligate FW stingray *Potamotrygon circularis* exhibited a minimum behavioral threshold of $120\,\mu V\,cm^{-1}$ whereas Szamier and Bennett (Szamier and Bennett, 1980) reported the threshold sensitivity of *Potamotrygon* to be 1 mV. Whitehead (Whitehead, 2002) reported that bull sharks initiated all responses from >15 cm but did not report a maximum detection distance or behavioral threshold sensitivity. Although the sensitivity of the FW stingrays in this study is greater than these other species, it is still several orders of magnitude lower than any other marine elasmobranch.

The differences in sensitivity between the BSW and the FW stingrays can be partially attributed to the electrical properties of the water in these very distinct environments. Fig. 6 illustrates the voltage equipotentials of a dipole modeled in both seawater and FW using the same parameters as in this study (i.e. $8\,\mu A$ applied current, 1 cm dipole opening separation, seawater resistivity of $19\,\Omega\,cm$ and FW resistivity of $2026\,\Omega\,cm$). Each equipotential represents an order of magnitude difference in both seawater and FW treatments. The model clearly illustrates that the voltage decreases much more dramatically with distance from the source in seawater compared with FW. For the maximum demonstrated detection distance (~40 cm) the electric field decreases by five orders of magnitude in seawater compared with only three orders of magnitude for that same distance in FW (Fig. 6A). The electric field strength drops much more quickly with distance in seawater (Fig. 6B). In essence, the electric field is being grounded in seawater whereas the electric field maintains its strength over a greater linear distance in the resistive FW environment, resulting in a much smaller voltage change with distance. So although the absolute voltage is greater in FW, the slope is much smaller. Therefore the relevant stimulus to the electrosensory system may be the relative change in voltage, rather than a minimum threshold.

The modeled dipole illustrated in Fig. 6 demonstrates that the electric field is directly proportional to resistivity (Kalmijn, 1982). In this study, the difference in mean resistivity between the saltwater ($\rho=19\,\Omega\,cm$) and FW ($\rho=2026\,\Omega\,cm$) treatments was approximately $100\times$, which should result in a proportional $100\times$ difference in electric field sensitivity. However, median electrosensitivity was more than $200\times$ greater in the BSW treatments ($6.0\,nV\,cm^{-1}$) than in the FW ($1.4\,\mu V\,cm^{-1}$) treatments. The disparity indicates that the reduced sensitivity in FW is not solely due to the electrical properties of the water but is due in part to biological differences when detecting electric fields in FW *vs* BSW environments.

Unlike the obligate FW Potamotrygonid stingrays, which have undergone dramatic morphological changes to their electrosensory system, the euryhaline elasmobranchs retain a marine electrosensory morphology characterized by long subdermal canals. The long canals have been convincingly argued to act like an antenna and provide the ampullae with the voltage difference along the length of the canals, at least in a marine environment (Brown et al., 2005). This is based upon a calculation of the path resistance along the length of a canal, which is much higher than the resistance between epidermal pores on the surface of the body. Using the same parameters and equations as Brown et al. (Brown et al., 2005), the canal resistance was calculated to be $160\times$ greater than the pore-to-pore resistance in seawater; the same value they achieved. However, in FW with a resistivity of $2000\,\Omega\,cm^{-1}$, the canal resistance is only slightly greater ($1.6\times$) than the pore-to-pore resistance. This may effectively reduce the typical antenna-like role and allow the canals to function as a cable and conduct the pore

voltage to the ampullae. However, this calculation is predicated upon the glycoprotein gel in the canals possessing the same resistivity in both FW and seawater environments – an assumption that remains to be tested. If this assumption is correct, the reduced ability of the ampullary system to function in FW could account for the observed reduction in sensitivity. The cable-like function of the canals in FW reduces the contrast in signals detected by the hundreds of electroreceptors across the sensory array (Kalmijn, 1974; Brown, 2002). Furthermore, the high external resistivity of FW results in a transcutaneous voltage difference, which effectively diminishes the demonstrated advantage of the varied ampullary canal geometry (Kalmijn, 1974; Camperi et al., 2007).

The reduction in sensitivity of the Atlantic stingray's electrosensory system in FW does not necessarily equate to a decrease in function. All FW stingrays were able to detect, localize and successfully bite at the prey-simulating stimulus, some from distances in excess of 15 cm. The FW population of Atlantic stingrays in the SJR completes their entire life cycle in FW (Johnson and Snelson, 1996), which represents the strongest evidence that the electrosensory system continues to function effectively in FW.

Despite their permanent exposure to FW over the past 100,000 years (Cook, 1939), the SJR stingrays did not demonstrate an enhanced electrosensitivity in FW, nor did they exhibit reduced sensitivity when reintroduced to higher salinities. Stingrays from both populations responded similarly to the prey-simulating stimulus when tested in similar salinities, regardless of from where they were collected in the wild. The plasticity of this sensory system to function in such diverse environments demonstrates its adaptive significance.

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