CSIRO PUBLISHING

Marine and Freshwater Research, 2015, **66**, 1027–1036
http://dx.doi.org/10.1071/MF14354

Electroreception in the obligate freshwater stingray, *Potamotrygon motoro*

Lindsay L. Harris[A], *Christine N. Bedore*[B] *and Stephen M. Kajiura*[A,C]

[A]Department of Biological Sciences, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33431, USA.
[B]Biology Department, Duke University, Durham, NC 27708, USA.
[C]Corresponding author. Email: kajiura@fau.edu

Abstract. Elasmobranch fishes use electroreception to detect electric fields in the environment, particularly minute bioelectric fields of potential prey. A single family of obligate freshwater stingrays, Potamotrygonidae, endemic to the Amazon River, demonstrates morphological adaptations of their electrosensory system due to characteristics of a high impedance freshwater environment. Little work has investigated whether the reduced morphology translates to reduced sensitivity because of the electrical properties of freshwater, or because of a marine-tuned sensory system attempting to function in freshwater. The objective of the present study was to measure electric potential from prey of *Potamotrygon motoro* and replicate the measurements in a behavioural assay to quantify *P. motoro* electrosensitivity. Median orientation distance to prey-simulating electric fields was 2.73 cm, and the median voltage gradient detected was 0.20 mV cm^{-1}. This sensitivity is greatly reduced compared with marine batoids. A euryhaline species with marine-type ampullary morphology was previously tested in freshwater and demonstrated reduced sensitivity compared with when it was tested in seawater (0.2 μV cm^{-1} *v.* 0.6 nV cm^{-1}). When the data were adjusted with a modified ideal dipole equation, sensitivity was comparable to *P. motoro*. This suggests that the conductivity of the medium, more so than ampullary morphology, dictates the sensitivity of elasmobranch electroreception.

Additional keywords: batoid, bioelectric fields, conductivity, passive electrosense.

Received 9 November 2014, accepted 17 January 2015, published online 9 April 2015

Introduction

Aquatic predators rely on a suite of sensory modalities to successfully detect and localise their prey. The physical properties of the aquatic medium dictate how stimuli are transmitted. For example, salinity directly correlates with conductivity and, thus, has a profound effect on the propagation of bioelectric fields.

Aquatic organisms produce an electric potential because of differences in ion concentration between their tissues and the external environment. Although the skin is relatively impermeable to ions, ion leakage occurs across mucus membranes in the mouth, gills and cloaca (Kalmijn 1972, 1974). Ventilatory activity modulates the electric potential, which provides spatial and temporal information about the source of the stimulus.

Marine elasmobranchs can detect bioelectric fields at magnitudes as small as a billionth of a volt per centimetre (Kajiura and Holland 2002). This sensitivity is especially advantageous for the detection of cryptic prey buried in the substrate or in environments in which other sensory systems are hindered, such as turbid waters. Most studies of passive electroreception are limited to marine elasmobranchs, despite the widespread presence of euryhaline and stenohaline species (McGowan and Kajiura 2009).

Stingrays within the family Potamotrygonidae are the only true stenohaline freshwater elasmobranchs and they are endemic to the Amazon River basin (Lovejoy *et al.* 2006). The potamotrygonid stingrays demonstrate a suite of morphological and physiological adaptations to a freshwater existence, including thickening of the dermis to aid in osmoregulation by providing a barrier to ion leakage (Szabo *et al.* 1972). The thickened skin concomitantly forms a high-resistance electrical barrier between the internal environment of the stingray's tissues and the external freshwater environment (Szabo *et al.* 1972). As a result, the electrosensory ampullary tubules need only be transcutaneous to detect environmental electric stimuli. In contrast to their marine relatives, the potamotrygonid stingrays possess microscopic electrosensory pores and short canals that lead to single ampullae embedded in the dermis (Szabo *et al.* 1972; Szamier and Bennett 1980). Collectively, high skin resistance and small, superficial ampullary organs possibly maintain functionality of the electrosensory system (Szabo *et al.* 1972). Previous studies have suggested that *Potamotrygon* spp. demonstrate reduced sensitivity to bioelectric fields compared with their marine counterparts (Szabo *et al.* 1972; Szamier and Bennett 1980). Although this early research confirmed the presence of the electrosensory system in a freshwater elasmobranch, its importance in an ecological context was not well documented.

Table 1. Morphometrics of study species
DL, disc length; TL, total length

Order	Family	Species	*n*	TL or DL range (cm)	Mass range (g)
Siluriformes	Loricaridae	*Hypostomus plecostomus*	6	6.3–7.8 (TL)	2.3–4.7
Characiformes	Characidae	*Colossoma bidens*	6	6.0–9.0 (TL)	3.1–9.9
Perciformes	Cichlidae	*Astronotus ocellatus*	6	6.0–8.0 (TL)	4.9–8.6
Myliobatiformes	Potamotrygonidae	*Potamotrygon motoro* (e-fields)	6	10.5–12.5 (DL)	68–113
		Potamotrygon motoro (behaviour)	7	12–15 (DL)	112–212

The goal of the present study was to quantify the functional capabilities of the electrosensory system of an obligate freshwater stingray that inhabits an electrically resistive medium and possesses a reduced electrosensory morphology. To achieve this, the objectives were to (1) quantify the electric potential of the obligate freshwater stingray, *Potamotrygon motoro* Garman 1877, and its teleost prey items, (2) mathematically model voltage decay in freshwater, and (3) quantify behavioural sensitivity of *P. motoro* to prey-simulating electric fields and compare this sensitivity to marine sister taxa.

Materials and methods

Voltage and frequency determination

An electrophysiological technique was employed to quantify the electric potentials (i.e. voltage) produced by the obligate freshwater stingray, *P. motoro*, its representative teleost prey items (Almeida *et al.* 2010) and a bioelectric field generator (BFG). These data were then used to determine an appropriate prey-simulating electric stimulus for use in the subsequent behavioural assay.

Animal acquisition and maintenance

Six similar-sized individuals each of *P. motoro* and three teleost prey species were acquired from local aquarium suppliers (Table 1). All fish were housed in temperature-controlled aquaria equipped with mechanical, chemical and biological filtration. Fish were housed in the laboratory for a maximum of 2 days and were not fed during that time. Electric potential was measured at least 12 h after acquisition.

Experimental setup

An acrylic experimental tank ($89 \times 43 \times 21$ cm) contained aerated, de-chlorinated freshwater with water chemistry similar to that of the holding tank (i.e. 25.0 ± 1°C and pH 7 ± 1). A 100-µm-tip recording electrode was built using an Ag–AgCl pellet within a 1.5-mm-diameter glass capillary tube filled with 3 M KCl solution (E45P-M15NH, Warner Instruments, Hamden, CT, USA). The electrode tip was placed <1 mm from the target location on the body and an identical reference electrode was placed in the corner of the experimental tank (see Fig. S1, available as Supplementary material for this paper). The output from the two electrodes was differentially amplified (DP-304, Warner Instruments) at 1000×, filtered (0.1 Hz–0.1 kHz, 60 Hz notch; DP-304, Warner Instruments & Hum Bug, Quest Scientific, North Vancouver, British Columbia, Canada), digitised at 1 kHz by using a Power Laboratory 16/30 model ML 880 (AD Instruments, Colorado Springs, CO, USA) and recorded using Chart Software (v.5, AD Instruments).

All teleost holding and experimental trials were conducted at Florida Atlantic University's Harbor Branch Oceanographic Institute (FAU–HBOI) in Fort Pierce, Florida, USA, or at the FAU Marine Sciences Laboratory in Boca Raton, Florida. Experimental and holding-tank conditions were identical at each location. Freshwater stingrays were maintained, and voltage measurements were conducted, at the Florida Fish and Wildlife Conservation Commission (FWC) Non-native Fish Laboratory in Boca Raton, Florida, or Ornamental Fish Distributors (OFD) in Miami, Florida.

Experimental protocol

Each individual teleost ($n = 6$ per species) was lightly anesthetised with a buffered solution of MS-222 (tricaine methanesulfonate buffered with sodium bicarbonate, Western Chemical Inc., Ferndale, WA, USA; 1 : 10 000–1 : 13 000 weight : vol (100–76.3 mg L^{-1})), to a level that minimised full body movements, but allowed natural ventilation. Fish were secured with Velcro tape to a vertical acrylic stage, which was attached to the submerged arm of a linear translation system (Newmark Systems Inc., eTrack-300 and NSC-1S, Rancho Santa Margarita, CA, USA) positioned adjacent to the tank. The tip of the recording electrode was placed sequentially at the following four locations along the body: mouth, opercular opening, middle of the body between the gill operculum and the tail (trunk) and the caudal peduncle (tail). The electric potential of each individual was recorded during three trials at the above locations along the body, and averaged to quantify mean electric potential for each species. To measure voltage decay from the mouth, the fish was moved in computer-controlled 1-cm increments away from the recording electrode with the linear translation system, until the signal was no longer distinguishable from background electrical noise in the tank. Mass (g) and total length (TL, cm) were recorded for each fish.

For the freshwater stingrays (3 males, 3 females), each individual was lightly anesthetised (buffered MS-222; 1 : 15 000 weight : vol (67 mg L^{-1})) in the experimental tank as described for the teleosts. Stingrays were secured to a plastic mesh horizontal stage submerged in the tank. Voltage was recorded at the following four equivalent locations along the body as described for teleosts: the mouth, first gill slit, middle of the body and midway down the tail on the ventral surface. An additional measurement was taken at the spiracle on the dorsal

surface. Mass (g), disc width (cm) and disc length (cm) were recorded for each stingray. This research was conducted in accordance with FAU IACUC Protocol #A09-20.

Frequency

In addition to electric potential, ventilatory frequency was recorded from the location that a predator would most likely detect an electric stimulus, namely, the mouth of the teleosts and the spiracle of the stingrays. A fast Fourier transform (FFT) was used to derive the fundamental frequency from the power spectrum of each species. FFTs were conducted on both bioelectric and background electrical signals. Mean fundamental frequency for each species was reported.

Statistical analyses

One-way ANOVA ($\alpha = 0.05$) followed by a Tukey *post hoc* test determined significant differences in frequency at the mouth within species, and also among species. These tests also determined significant differences in electric potential across the body within and among species. Regression analyses tested for relationships among voltage and frequency with length and mass. All statistical analyses were conducted using JMP statistical software (v.10.0, SAS Institute, Cary, NC, USA).

Voltage modelling

To confirm the electric-field characteristics of stimuli used in subsequent behaviour trials, the electric potential produced by a BFG was measured in the experimental tank used in the behavioural assay (described below). Environmental conditions during measurements were an average of conditions during the behaviour trials (i.e. $t = 28$°C, pH = 8, $\rho = 3022$ Ωcm). The tip of a glass electrode, identical to the ones used to measure voltage from prey items, was positioned <1 mm above the acrylic plate. An identical reference electrode was attached to the wall of the tank as far as possible from the stimulus.

An electrode placement guide was created using Adobe Illustrator (v.3, Adobe Systems Inc., San Jose, CA, USA) and printed onto an acetate sheet. It was then positioned on top of the dipole centre and secured to the plate. The placement guide was marked with distances of 1, 2, 3, 4, 5 and 10 cm with respect to the dipole centre and angles from 0 to 90° in 15° increments with respect to the dipole axis. A 2.2 μA stimulus was applied across a 1-cm dipole in the centre of the placement guide. A distance and angle were randomly paired on the placement guide and the voltage was measured at each location. All distance and angle combinations were measured three times and averaged to generate a mean voltage produced by the BFG. The voltage was plotted against distance at an angle of 0° with respect to the dipole axis. The voltage was also plotted as a function of angle from 0 to 90° at distances of 1, 2, 3, 4, 5 and 10 cm from the centre of the dipole.

Freshwater stingray behavioural sensitivity

An applied direct current (DC) that ranged from 1.2 to 4.0 μA was used in behavioural trials to replicate the approximate electric potential of the previously measured prey items (i.e. mean 0.70 mV, range 0.30–1.33 mV). Environmental conditions during experiments were as follows: temperature ranged from 27 to 29°C, pH ranged from 7.84 to 8.30, and resistivity ranged from 1153.40 to 3403.68 Ωcm. Average temperature, resistance and conductivity values were used to estimate the appropriate applied current to serve as a prey-simulating bioelectric stimulus (Bedore and Kajiura 2013).

Animal acquisition and maintenance

Seven juvenile *P. motoro* individuals were acquired from Ornamental Fish Distributors in Miami, Florida, and transported to the FWC Non-native Fish Research Laboratory where experimental trials took place (Table 1). Prior to and throughout the duration of the behaviour trials, two stingrays each were held in aquaria equipped with mechanical, chemical and biological filtration. Stingrays were fed to satiation daily on a diet of shrimp, fish and live blackworms. Experiments began after all stingrays were fully acclimated to the laboratory setting, indicated by feeding daily for a minimum of 1 week. During trials, stingrays were fasted for 2 days to ensure that they were motivated to respond to prey-simulating stimuli.

Experimental setup

To determine the sensitivity of *P. motoro* to bioelectric fields, a behavioural assay was employed following methods described in similar studies (Kajiura and Holland 2002; Kajiura 2003; Jordan *et al.* 2009; McGowan and Kajiura 2009). An opaque acrylic plate, 2 m in diameter, was placed on the bottom of a 3785-L fibreglass experimental tank at the FWC laboratory. A vertical plastic barrier attached to the surface of the plate restricted the experimental area to ~1.5 m in diameter. Four electric dipoles were equally spaced 50 cm apart on the plate (i.e. dipole array). Dipole electric fields were induced with a BFG (cf. Kajiura and Holland 2002) connected to four shielded underwater cables, which each terminated in a pair of gold-plated stainless-steel electrodes (18 AWG SO, LPIL-2, Teledyne Impulse, San Diego, CA, USA). The electrodes were each affixed to a 1.4-m-long, water-filled polyethylene tube mounted to the underside of the acrylic plate. They opened to the tank water through two 1-mm holes in the plate spaced 1 cm apart, which represents the approximate size of a dipole electric field produced by a prey item. A multimeter connected in series to the BFG monitored the applied current (see Fig. S2, available as Supplementary material for this paper). To ensure proper lighting for video analysis, four lamps equipped with incandescent 100-W bulbs were affixed to the sides of the tank to adequately illuminate the dipole array. To maintain a constant water temperature in the experimental tank, four 800-W submersible heaters were used, but were removed during trials to eliminate the potential for electrical interference.

Experimental protocol

A stingray pair was placed in the experimental tank and was allowed to acclimate for 1 h before experimental trials. To begin a trial, a food odour was introduced into the tank via an odour-delivery tube mounted to the centre of the acrylic plate. Once the stingrays were motivated to feed, as indicated by prey searching behaviour, the odour delivery ceased and one of the four electrode pairs was randomly activated. The three inactive dipoles served as controls. As a single stingray approached the

dipole, it made a turn towards the dipole and bit as if it encountered a prey item. Once the stingray bit at the active dipole, the stimulus was deactivated and another dipole was randomly activated on the plate. Trials were recorded with a HD video camera (Sony HDR-CX 260, Tokyo, Japan) mounted over the centre of the array. Each trial lasted a maximum of 60 min, depending on motivational state, and each stingray was tested a minimum of three times over a period of 2 weeks. This research was conducted in accordance with FAU IACUC protocol #A11-38 and FWC conditional non-native species permit EXOT-12-45.

Video analysis

Video trials were imported to a computer and edited with iMovie (v.7.1.4, Apple Computer, Inc., Cupertino, CA, USA). The frame in which an orientation to a dipole was initiated was exported as a still image for analysis. The distance from the centre of the dipole to the stingray's disc margin was measured with image-analysis software (ImageJ, National Institutes of Health, Bethesda, MD, USA). In addition, the angle described by the point of orientation with respect to the dipole axis was measured in the same manner. A dipole field equation was used to calculate the electric field (i.e. voltage gradient; V cm^{-1}) at the point of orientation, thus providing a measure of sensitivity (Kalmijn 1982; Kajiura and Holland 2002), as follows:

$$E = \frac{\rho I d}{\pi r^{x}}(\cos\theta) \quad (1)$$

where ρ is the resistivity of freshwater (1153.40–3403.68 Ωcm), I is the applied current (1.2−4.0 μA), d is the dipole separation distance (1 cm), r is the orientation distance (measured at the disc margin; cm), x is the power of the derived voltage gradient (2.22), and θ is the orientation angle with respect to the dipole axis (degrees).

Results

Voltage and frequency determination

Electric potential and ventilatory frequency were measured from six individuals of juvenile obligate freshwater stingray *P. motoro*, and three species of their common teleost prey items (Table 1). Power spectrum analysis was used to quantify fundamental frequency (reported), secondary harmonics and background electrical noise for each species (following Bedore and Kajiura 2013). Ventilatory frequency differed among the four species (Fig. 1). Mean (±s.d.) frequency produced by the pleco catfish, *Hypostomus plecostomus* (3.49 ± 0.54 Hz), was significantly higher than those produced by the other species. The tiger Oscar, *Astronotus ocellatus* (1.01 ± 0.33 Hz), and *P. motoro* (0.67 ± 0.22 Hz) produced the lowest frequencies (ANOVA; $F_{3,20} = 38.64$, $P < 0.001$). The red-bellied pacu, *Colossoma bidens*, produced a ventilatory frequency intermediate between the lowest and highest frequencies among the species (2.16 ± 0.65 Hz). There was no relationship between frequency and total length among all species (regression; $R^2 < 0.55$, $p > 0.05$). Among teleost species, there was no relationship between frequency and total length (regression; $R^2 = 0.005$, $F_{1,16} = 0.08$, $P = 0.78$); however, there was a slight positive linear relationship between frequency and mass (regression; $R^2 = 0.22$, $F_{1,16} = 4.63$, $P = 0.05$). Only in the stingrays was frequency predicted by mass (within species regression; $R^2 = 0.75$, $F = 11.84$, $P = 0.03$ for stingrays; $R^2 < 0.20$, $P > 0.05$ for all other species).

Electric potential was measured at the mouth, gill opercula, trunk and tail of each fish (Fig. 2). Additional electric-potential measurements were recorded from the spiracle of *P. motoro*, but were omitted from analysis to facilitate comparison with teleosts. The spiracle produced a mean (± s.d.) electric potential of 1.59 ± 0.98 mV. Among all species, electric potential at the anterior portion of the body (i.e. mouth and gills) was significantly greater than that at the posterior portion of the body (i.e. trunk and tail, ANOVA; $F_{3,92} = 10.89$, $P < 0.001$). Although this general trend was seen in all species, it was not a significant difference within all species. There was no relationship between electric potential and total length or mass among or within species (Table 2).

Electric potential was measurable up to 5 cm from the mouth of the teleosts, and decreased dramatically at distances beyond 1 cm from this location (Fig. 3). There was a 78% decrease in voltage from the mouth to 1 cm away in *C. bidens*. *Hypostomus plecostomus* and *A. ocellatus* demonstrated a 91% decrease in voltage 1 cm from the mouth.

Voltage modelling

A bioelectric-field generator (BFG) was used to produce a prey-simulating stimulus based on the measured electric potential of

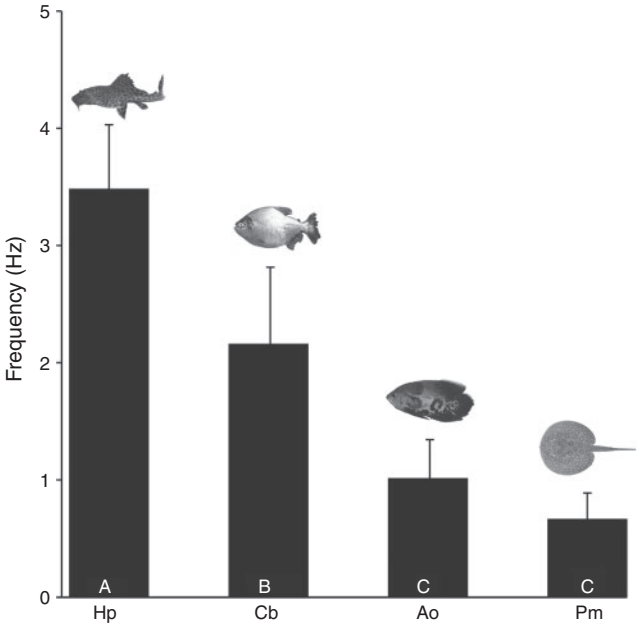

Fig. 1. Dominant ventilatory frequencies (mean ± s.d.) exhibited by the obligate freshwater stingray, *Potamotrygon motoro*, and its common teleost prey. Frequency was measured at the mouth of teleosts and at the spiracle of stingrays. The pleco catfish, *Hypostomus plecostomus* (Hp), produced the highest ventilatory frequency at 3.49 Hz, whereas *P. motoro* (Pm) produced the lowest frequency at 0.67 Hz. Vertical bars that share the same letter do not differ significantly. Cb, *Colossoma bidens*; Ao, *Astronotus ocellatus*.

the teleost prey items. The measured electric potential from the BFG was largely consistent with a theoretical ideal dipole in half space (Fig. 4). A 2.2-μA current generated a maximum electric potential of 0.93 mV at 1 cm along the dipole axis. Voltage decreased with distance as a power function ($V \propto r^{-1.22}$) and became indistinguishable from background noise at distances greater than 10 cm from the dipole centre at all angles (Fig. 4*a*). Electric potential varied as a cosine function at angles less than 75° (Fig. 4*b*). At angles greater than 75°, voltage did not decrease as precipitously with increasing angle as predicted by the model. Although the measured values reflected a trend similar to theoretical voltage decay in saltwater (i.e. $V \propto r^{-2}$; Kalmijn 1982; Kajiura and Fitzgerald 2009), the measured electric potential decreased less rapidly in freshwater. On the basis of these data, the ideal dipole equation (IDE) was modified to more accurately describe voltage decay with distance in freshwater. The exponent of the first-order derivative of the standing voltage decay from the dipole centre ($E \propto r^{-2.22}$) replaced the inverse cube in the original IDE modelled for seawater (Kalmijn 1982; Kajiura and Holland 2002).

Freshwater stingray behavioural sensitivity

Stingrays demonstrated 365 total interactions with the active dipole, defined as any instance that a stingray approached within ~20 cm from the stimulus (distance based on previous studies;

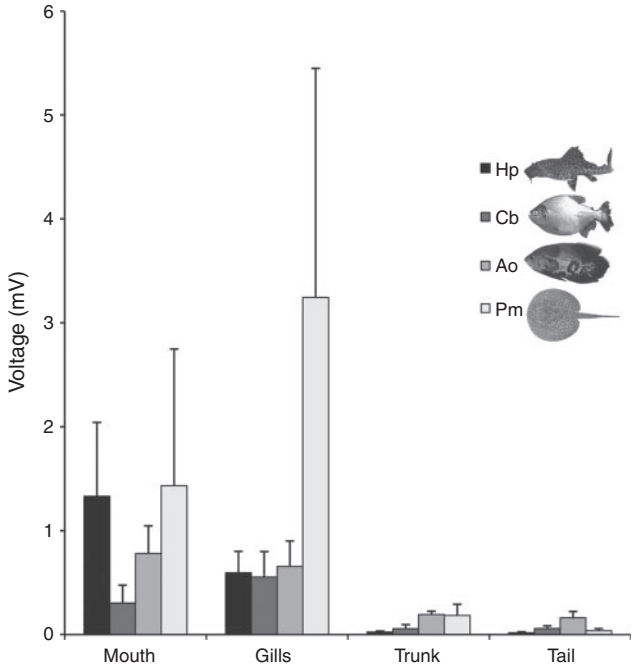

Fig. 2. Electric potential (mean ± s.d.) measured at several body locations on *Potamotrygon motoro* and its teleost prey. Species did not demonstrate significant differences in measured voltage at the mouth, but electric potential at the gills of *P. motoro* (Pm) was significantly larger than for the teleosts. Among species, electric potential at the posterior portion of the body (trunk, tail) was significantly smaller than that at the anterior portion (mouth, gills). Hp, *Hypostomus plecostomus*; Cb, *Colossoma bidens*; Ao, *Astronotus ocellatus*.

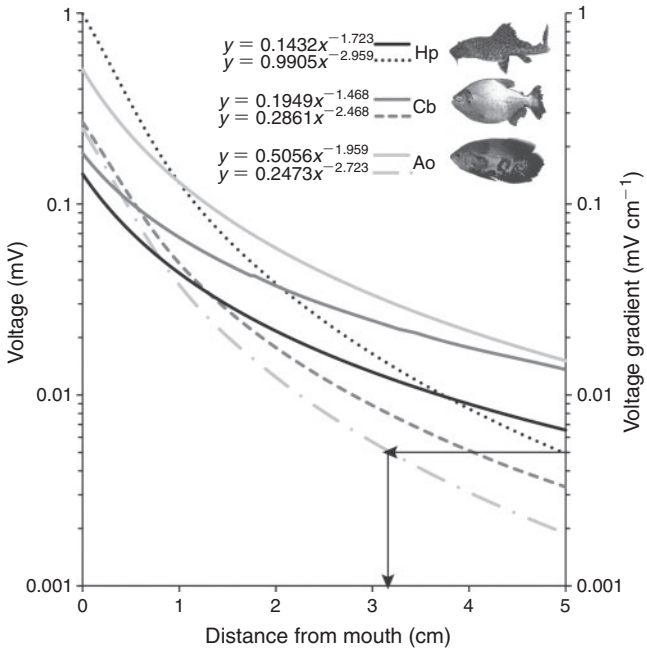

Fig. 3. Voltage-decay characteristics of teleost prey items of *Potamotrygon motoro*. Electric potential was measured up to 5 cm away from the mouth of each species and decreased with distance as a power function (solid lines). The dotted lines represent the voltage gradient calculated as the derivative of the measured voltage (power functions included in the figure). On the basis of a maximum demonstrated sensitivity by *P. motoro* (Pm) of 0.005 mV cm^{-1} (horizontal arrow), *Astronotus ocellatus* (Ao) would be within a detection distance at 3.2 cm (vertical arrow; shown in figure). *Hypostomus plecostomus* (Hp) and *Colossoma bidens* (Cb) would be in detection distance at 5.0 and 4.0 cm respectively (not shown).

Table 2. One-way ANOVA with Tukey *post hoc* test outputs (left), and regression analyses (right)

Within species, body locations connected by the same letter do not differ significantly in electric potential; regression analyses were used to determine body size (total length [TL], mass) effects on electric potential within a species

Species	$F_{3,20}$, P	Mouth	Gills	Trunk	Tail	TL R^2, P	Mass R^2, P
Hypostomus plecostomus	16.99, 0.0001	A	B	B	B	0.04, 0.3484	0.06, 0.3177
Colossoma bidens	14.59, 0.0001	B	A	C	BC	0.02, 0.4967	0.02, 0.5069
Astronotus ocellatus	17.86, 0.0001	A	A	B	B	0.03, 0.4517	0.02, 0.4830
Potamotrygon motoro	8.01, 0.0011	AB	A	B	B	0.10, 0.1409	0.10, 0.1305

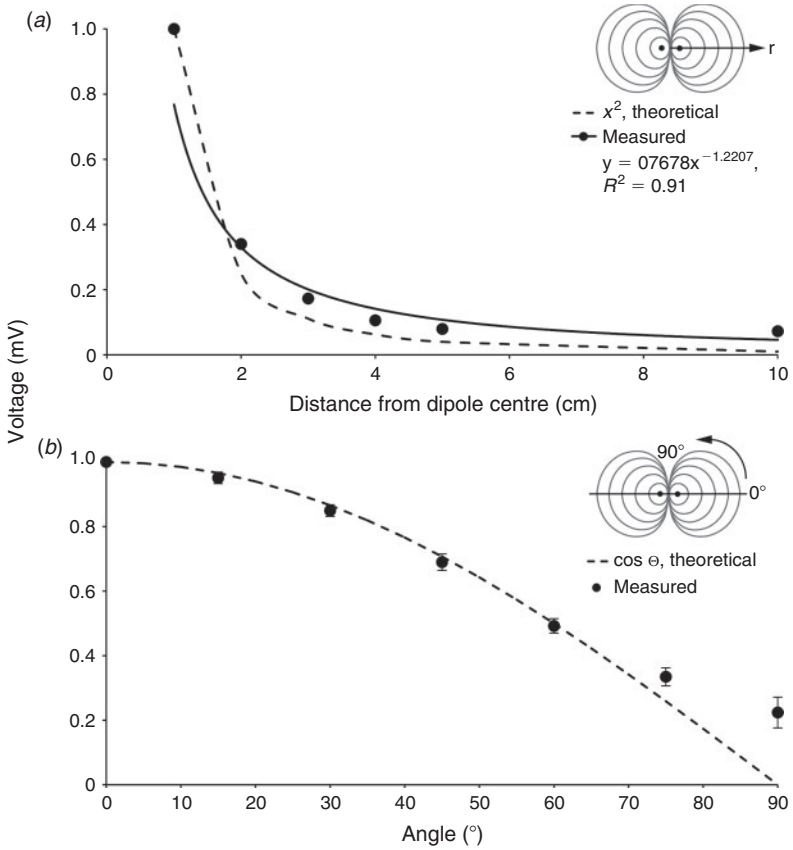

Fig. 4. Voltage modelling of the signal output from the bioelectric field generator. (*a*) Voltage decreased with distance as a power function (solid line) along the dipole axis. Measured values (points; mean ± s.e.) followed a similar trend but were not matched with theoretical values (dotted lines) based on an ideal dipole that decreases as an inverse square with distance. (*b*) Voltage decreased as a cosine function at angles less than 75°. Measured values (points; mean at distances of 1, 2, 3, 4, 5 and 10 from the dipole centre ± s.e.) closely match theoretical values (dotted line) of a typical cosine dependence of a dipole in half space.

Jordan *et al.* 2009; McGowan and Kajiura 2009; Bedore *et al.* 2014). Of the total interactions, 186 of these produced a change in trajectory towards the active dipole and subsequent bite at the dipole centre (i.e. orientation). Of the 186 interactions, 42.5% involved a single turn towards the dipole centre, followed by a bite (mean orientation distance of 3.48 cm). Spiral tracking was observed in 15.6% of the interactions, where the stingray made several turns along the voltage equipotentials towards the dipole centre, until initiating a final bite at the target (Kajiura and Holland 2002; Jordan *et al.* 2009). In total, 20% of the 186 orientations were omitted from further analysis because of factors that violated the criteria of the observer. These included orientations greater than 75° to the dipole centre, based on previous electric-potential modelling (i.e. measured voltage deviated from a theoretical cosine at larger angles, and therefore could not be used as a reliable predictor of voltage at those angles), the orientation was initiated too close to the dipole centre to be measured accurately, or it could not be determined if the orientation was an intentional or random turn towards the active dipole. Interactions (e.g. physical contact or path obstruction of one individual by another) between a stingray pair at the active dipole, although rare, were also omitted from analysis.

The maximum orientation distance occurred at 10.62 cm from the dipole centre that yielded a calculated sensitivity of 0.005 mV cm^{-1}. The majority of orientations (91.22%; $n = 135$) were initiated to stimuli of <1 mV cm^{-1} and 37.84% ($n = 56$) of these occurred at voltage gradients of <0.1 mV cm^{-1} (Fig. 5*a*). The median demonstrated sensitivity was 0.20 mV cm^{-1} ± 1.05 s.d. Most orientations were initiated <3 cm from the dipole centre (67.57%; $n = 100$; Fig. 5*b*). The median orientation distance was 2.73 cm ± 2.29 s.d. from the dipole centre. Orientation distance and angle demonstrated a weak relationship (regression; $R^2 = 0.03$, $F_{1,147} = 4.10$, $P = 0.05$).

Calculating detection distance on the basis of the derived voltage gradient

The power functions that described voltage decay with distance from the mouth of the teleosts were used to calculate the first-order derivative, yielding an equation for the voltage gradient (shown in Fig. 3). A maximum demonstrated behavioural sensitivity of 0.005 mV cm^{-1} was applied to the equations to

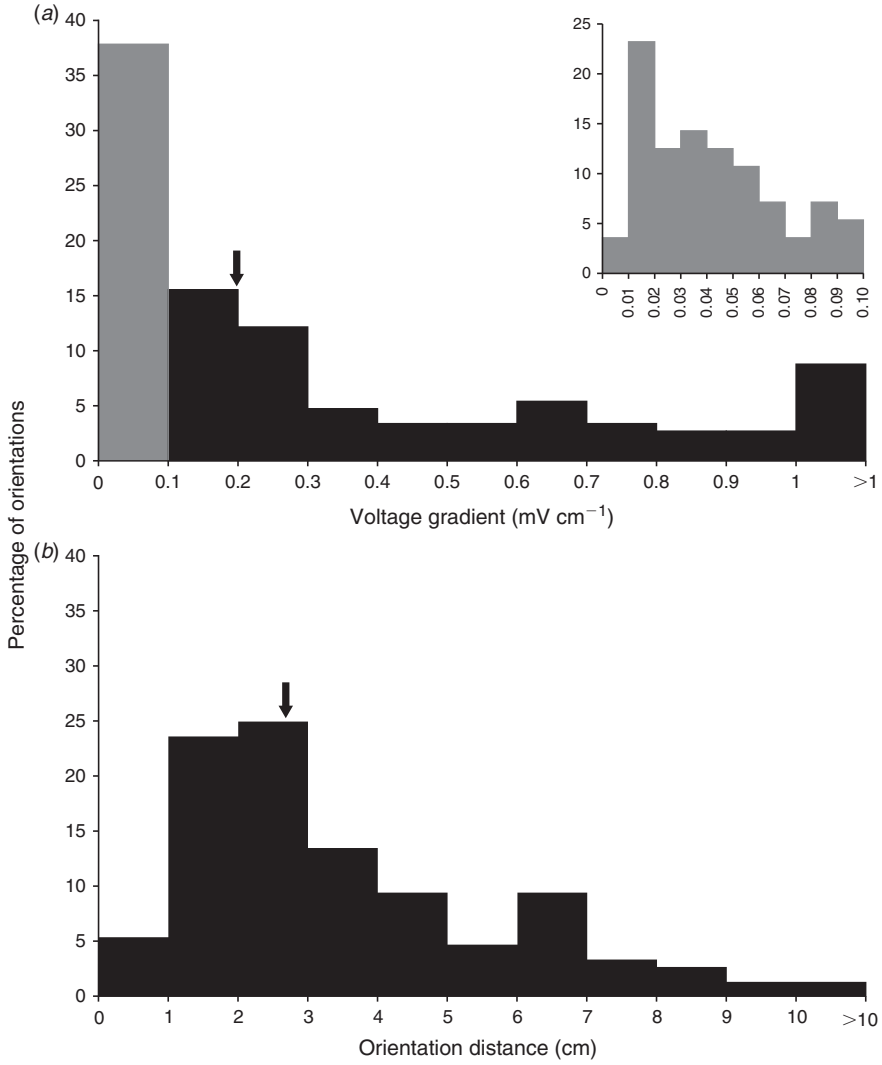

Fig. 5. Histograms of the percentage of orientations to the active dipole initiated by *P. motoro* during behavior trials. (*a*) The median voltage gradient that elicited a behavioral response was 0.20 mV cm^{-1} (arrow). Over 35% of orientations occurred at voltage gradients <0.1 mV cm^{-1} (inset: distribution of values within 0–0.1 mV cm^{-1}). The maximum demonstrated sensitivity by any individual stingray occurred at 0.005 mV cm^{-1}. (*b*) The median orientation distance was 2.73 cm (arrow) and most orientations occurred <3 cm from the dipole centre. The farthest orientation distance from the active dipole was 10.62 cm.

determine the estimated detection distance of each prey item by *P. motoro* (Fig. 3). *Hypostomus plecostomus* was calculated to be electrically detectable up to ~5.0 cm away, whereas *C. bidens* and *A. ocellatus* were calculated to be detectable at a maximum distance of 4.0 and 3.2 cm respectively.

Discussion

The present study is the first to empirically measure the bioelectric fields of various prey items and use those data to generate a prey-simulating stimulus to elicit a feeding response in an obligate freshwater elasmobranch. Modelling the bioelectric field enabled us to determine the electrosensitivity of the stingray *P. motoro* and compare the results to those of euryhaline and marine species.

Voltage and frequency determination

The specific characteristics of bioelectric fields, frequency, electric potential and voltage decay with distance, permit detection and localisation of individual prey items by an electroreceptive predator. These features were measured from the obligate freshwater stingray *P. motoro* and three of its common teleost prey items, so as to better understand bioelectric stimuli encountered by conspecifics and predators. Ventilation modulates the electric potential by periodically exposing the mucous membranes to the environment, which provides predators with temporal information. Elasmobranchs are sensitive to frequencies up to 20 Hz, with peak sensitivity to stimuli <2 Hz (Tricas *et al.* 1995; Tricas and New 1998). Common prey items of elasmobranchs and conspecifics both fall within this range

(Taylor *et al.* 1992; Almeida *et al.* 2010) and the frequencies measured from all species in the present study were similar to those measured in other aquatic organisms (Haine *et al.* 2001; Bedore and Kajiura 2013).

Among the teleost prey, electric potential at the mouth and gills greatly exceeded that at the trunk and tail. This result is not surprising, given that ions such as Na^+, K^+ and Cl^- are exchanged at mucous membranes in the mouth and gill during respiration and osmoregulation (Robertson 1953; Foskett *et al.* 1983). The interaction between these ions and the surrounding ion-poor environment creates a voltage gradient that emanates from the prey item (Kalmijn 1972). Electric potential in freshwater organisms is typically greater than that in marine species (Kalmijn 1988). Electric potential in most marine invertebrates ranges from 0.001 to 0.1 mV, and in teleosts it ranges from 0.02 to 0.3 mV (Haine *et al.* 2001; Bedore and Kajiura 2013). In the present study, electric potential at the mouth of *Hypostomus plecostomus* exceeded 1 mV, supporting previous literature on freshwater prey (Peters and Bretschneider 1972; Taylor *et al.* 1992). Different osmoregulatory strategies may contribute to some variation in electric potential between freshwater and marine organisms because of the ion disparity that occurs between the internal and external environment in the different media.

Voltage was measured from the same body locations in all species, with an additional measurement taken at the spiracle of *P. motoro*. Whereas the electric potential at the mouth of the stingrays fell within the range produced by the teleosts, the electric potential at the gills was approximately five times greater than the mean electric potential produced at the teleost gills. Freshwater teleosts and elasmobranchs both utilise the gills as a primary source of internal ion regulation (Ballantyne and Robinson 2010). In addition, both groups excrete nitrogenous waste in the form of predominantly ammonia and small amounts of urea (Wood *et al.* 2002). Unlike the teleosts, freshwater stingrays demonstrate high rates of ammonia excretion (e.g. rainbow trout, *Oncorhynchus mykiss*, 263 μmol kg^{-1} h^{-1}, and *Potamotrygon* sp., 507 μmol $kg^{-1}h^{-1}$; for review see Ballantyne and Robinson 2010). This larger waste excretion by *Potamotrygon* sp. may contribute to the difference in voltage seen in the present study between the two groups. *Potamotrygon motoro* produced voltage at the anterior portion of the body three orders of magnitude greater than that produced by marine batoids (Bedore and Kajiura 2013).

Voltage (mV) decreases rapidly as an inverse power function with distance from the source and the decay rate is dictated by the permittivity of the medium. This change over distance creates a voltage gradient (mV cm^{-1}) that electroreceptive predators use to localise an electric stimulus. Voltage decay from the mouth of the teleosts was measured to better understand how an elasmobranch detects the bioelectric field of its prey. Voltage rapidly decreased 1 cm from the source similar to other studies of marine species (Haine *et al.* 2001; Bedore and Kajiura 2013). Using a maximum demonstrated sensitivity of 0.005 mV cm^{-1} in the behavioural assay, it was determined that *P. motoro* should be able to detect a teleost prey item from a distance of up to 5.0 cm. This derived detection distance fell within the range of orientations demonstrated by *P. motoro* in behaviour trials using a prey-simulating stimulus (i.e. over 75% of orientations occurred at distances of 5 cm or less from the dipole centre, see below).

Voltage modelling

Because of the dissimilar electrical properties of freshwater and saltwater, it was necessary to validate that the BFG used in previous behavioural experiments in saltwater was suitable for use in freshwater. Empirical measurements confirmed that the BFG produced an electric potential in freshwater that conformed to the predicted values of a dipole electric field in half space. The electric potential varied as a cosine function with angle at angles less than 75° from the dipole axis. At angles close to normal, electric potential could not be accurately modelled with the cosine function. The voltage at these angles is small so measurement error is exaggerated and likely accounts for the disparity. When the recording electrode crossed 90°, there was a predicted change of sign from positive to negative, as observed in other studies (Kajiura and Fitzgerald 2009).

Although electric potential in freshwater varied as a cosine function, as in saltwater, the decay rate (dictated by the exponent) differed between the two media. Previous empirical measurements reported that electric potential decays as an inverse square with a derived voltage gradient of r^{-3} in saltwater (Kajiura and Fitzgerald 2009). In the present study, empirically measured electric potential produced by the BFG decayed with a relationship of $r^{-1.22}$, which yielded a derived voltage gradient of $r^{-2.22}$. These values were smaller than previously reported using the same BFG in saltwater ($r^{-1.95}$ and $r^{-2.95}$; Bedore and Kajiura 2013). Therefore, for calculations of electrosensitivity in the present study, the empirically measured voltage decay was employed.

An assumption of the model used to calculate electric-field strength is that the orientation distance (r) greatly exceeds the dipole separation distance (d; in this case $d = 1$ cm; Kalmijn 1982). Almost all of the orientations towards the dipole were initiated from a distance <10 cm from the dipole centre. As a result, the distance assumption is violated. However, because we empirically measured the electric field around the dipole and did not simply rely on a general model, we have reasonable confidence in our results.

Freshwater stingray behavioural sensitivity

Electrosensitivity to bioelectric stimuli has been well documented in marine and euryhaline elasmobranch species, but is relatively untested in freshwater species. The present study confirmed that an obligate freshwater elasmobranch uses its electrosensory system to detect and localise prey in a manner similar to marine batoids, albeit with greatly reduced sensitivity. *Potamotrygon motoro* demonstrated maximum and median behavioural sensitivity that was respectively four and five orders of magnitude lower than marine stingrays (Jordan *et al.* 2009; McGowan and Kajiura 2009; Bedore *et al.* 2014). For example, *P. motoro* initiated the greatest number of orientations to the prey-simulating dipole from a distance of 3 cm or less and the maximum orientation distance to the source was 10.62 cm, which is substantially smaller than that for marine and euryhaline batoids (24–77 cm; Jordan *et al.* 2009; McGowan and Kajiura 2009; Wueringer *et al.* 2012; Bedore *et al.* 2014).

The high ion content of seawater facilitates electrical conductivity, which results in a precipitous decrease in voltage with distance. In contrast, an electric stimulus in freshwater dissipates less rapidly and thus the voltage propagates farther than in saltwater. Because elasmobranch electroreceptors detect the voltage change with distance (i.e. a voltage gradient, V m^{-1}), rather than absolute voltage, the steeper slope produced by a charge in seawater can be detected at a greater distance than the shallower slope produced in freshwater (McGowan and Kajiura 2009). During the behaviour experiments, stingrays had to be within 10 cm of the dipole to detect the stimulus and orient towards it, possibly as a consequence of the physical constraints of the medium.

The electrosensitivity of *Daysatis sabina*, a euryhaline ray with marine-type ampullary morphology, was reported as three orders of magnitude lower in freshwater than saltwater, with a corresponding decrease in orientation distance (McGowan and Kajiura 2009). It was undetermined whether the decreased sensitivity was attributable to unsuitable morphology to the freshwater electrical environment or to the electrical properties of freshwater. The electrosensitivity calculated in that study used the standard IDE for saltwater, which applies a voltage decay rate of r^{-3} (Kalmijn 1982; Kajiura and Holland 2002). On the basis of the empirically measured voltage in the present study, a voltage decay of $r^{-2.22}$ would be a better model for freshwater. When a voltage decay of $r^{-2.22}$ is applied to the *D. sabina* data, the calculated median and maximum sensitivity both decrease by an order of magnitude (to 0.01 and 0.003 mV cm^{-1}). The revised maximum sensitivity of *D. sabina* is comparable to the best response seen in *P. motoro* (0.005 mV cm^{-1}). This suggests that the electrosensitivity is a function of the electrical properties of the medium and not a constraint imposed by the marine-type ampullary morphology functioning in freshwater.

An apparently less sensitive electrosensory system than that of marine taxa should not be considered a detriment. Potamotrygonid stingrays exhibit adaptations that promote sensory integration to facilitate successful prey capture. Several species of freshwater stingray possess an increase in the length of the infraorbital lateral line canal located on the ventral surface near the mouth (Shibuya *et al.* 2010). This is considered to be a derived condition that may improve the accuracy of prey localisation when foraging, by increasing the surface area available to receive tactile cues by prospective prey at close range (i.e. mechanotactile hypothesis; Maruska and Tricas 1998). Freshwater stingrays commonly exhibit opportunistic feeding tactics when cruising the substrate, by undulating their disc to excavate cryptic prey (Garrone-Neto and Sazima 2009). When prey are detected using this technique, they are already at close range to the electroreceptors and electroreception is likely to be employed to guide the mouth to individual prey items trapped directly under the disc. The application of mechanotactile and electrosensory information in concert synergistically provides the stingray with greater localisation efficiency than does either sensory modality in isolation because sensitivity thresholds decrease when multiple sensory systems are integrated (Stein and Meredith 1993). This differs from marine species in which electro-orientation is initiated farther away from the source, so mechanotactile cues do not come into play in detection, but in localisation only. Compared with the marine environment, the freshwater environment is electrically 'noisy' (Peters and Bretschneider 1972; Kalmijn 1988), so differentiating local dipoles produced by prospective prey may prove difficult until close to the source.

Conclusions and future research

The present study empirically confirmed that a stenohaline elasmobranch utilises electroreception to detect and localise prey items in a similar fashion as marine species, despite reduced electrosensory morphology. Although considered generalistic in their feeding habits, some populations of *P. motoro* demonstrate ontogenetic shifts in diet from small invertebrates as juveniles to mobile fishes as adults (Almeida *et al.* 2010). Additional testing of adults using the same stimuli will provide insight into whether sensitivity changes throughout ontogeny. Electrosensitivity measurements using controlled manipulation of salinity (i.e. conductivity) or other water parameters often encountered in the Amazonian environment may also contribute to our understanding of how this unique marine-derived sensory system functions in an electrically unfavourable medium.

Acknowledgements

The authors thank K. Gestring and staff of the FWC Non-native Fish Laboratory for extensive use of their facilities during behaviour trials. A. Harris, D. Knott, V. Zollo and T. Gunn for experiment and husbandry support; P. S. Wills and K. Sorge for suggestions to improve this manuscript. This work was supported by the American Elasmobranch Society Donald R. Nelson Behaviour Research Award and an FAU academic achievement award.

References

Almeida, M. P., Lins, P. M. O., Charvet-Almeida, P., and Barthem, R. B. (2010). Diet of the freshwater stingray *Potamotrygon motoro* (Chondrichthyes: Potamotrygonidae) on Marajó Island (Pará, Brazil). *Brazilian Journal of Biology* **70**, 155–162. doi:10.1590/S1519-69842010000100022

Ballantyne, J. S., and Robinson, J. W. (2010). Freshwater elasmobranchs: a review of their physiology and biochemistry. *Journal of Comparative Physiology. B. Biochemical, Systemic, and Environmental Physiology* **180**, 475–493. doi:10.1007/S00360-010-0447-0

Bedore, C. N., and Kajiura, S. M. (2013). Bioelectric fields of marine organisms: voltage and frequency contributions to detectability by electroreceptive predators. *Physiological and Biochemical Zoology* **86**, 298–311. doi:10.1086/669973

Bedore, C. N., Harris, L. L., and Kajiura, S. M. (2014). Behavioral responses of batoid elasmobranchs to prey-simulating electric fields are correlated to peripheral sensory morphology and ecology. *Zoology* **117**, 95–103. doi:10.1016/J.ZOOL.2013.09.002

Foskett, J. K., Bern, H. A., Machen, T. E., and Conner, M. (1983). Chloride cells and the hormonal regulation of teleost fish osmoregulation. *The Journal of Experimental Biology* **106**, 255–281.

Garrone-Neto, D., and Sazima, I. (2009). Stirring, charging, and picking: hunting tactics of potamotrygonid rays in the upper Paraná River. *Neotropical Ichthyology* **7**, 113–116. doi:10.1590/S1679-62252009000100015

Haine, O. S., Ridd, P. V., and Rowe, R. J. (2001). Range of electrosensory detection of prey by *Carcharhinus melanopterus* and *Himantura granulata*. *Marine and Freshwater Research* **52**, 291–296. doi:10.1071/MF00036

Jordan, L. K., Kajiura, S. M., and Gordon, M. S. (2009). Functional consequences of structural differences in stingray sensory systems. Part II: electrosensory system. *The Journal of Experimental Biology* **212**, 3044–3050. doi:10.1242/JEB.028738

Kajiura, S. M. (2003). Electroreception in neonatal bonnethead sharks, *Sphyrna tiburo*. *Marine Biology* **143**, 603–611.

Kajiura, S. M., and Fitzgerald, T. P. (2009). Response of juvenile scalloped hammerhead sharks to electric stimuli. *Zoology (Jena, Germany)* **112**, 241–250. doi:10.1016/J.ZOOL.2008.07.001

Kajiura, S. M., and Holland, K. N. (2002). Electroreception in juvenile scalloped hammerhead and sandbar sharks. *The Journal of Experimental Biology* **205**, 3609–3621.

Kalmijn, A. J. (1972). Bioelectric fields in sea water and the function of the ampullae of Lorenzini in elasmobranch fishes. In 'SIO Oceanography'. (Ed. SIO Reference 72–83.) pp. 1–21. (Scripps Institution of Oceanography, University of California: San Diego, CA.)

Kalmijn, A. J. (1974). The detection of electric fields from inanimate and animate sources other than electric organs. In 'Handbook of Sensory Physiology, Vol. 3'. (Ed. A. Fessard.) pp. 147–200. (Springer-Verlag: New York.)

Kalmijn, A. J. (1982). Electric and magnetic field detection in elasmobranch fishes. *Science* **218**, 916–918. doi:10.1126/SCIENCE.7134985

Kalmijn, A. J. (1988). Detection of weak electric fields. In 'Sensory Biology of Aquatic Animals'. (Eds J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga.) pp. 151–186. (Springer-Verlag: New York.)

Lovejoy, N. R., Albert, J. S., and Crampton, W. G. R. (2006). Miocene marine incursions and marine/freshwater transitions: evidence from Neotropical fishes. *Journal of South American Earth Sciences* **21**, 5–13. doi:10.1016/J.JSAMES.2005.07.009

Maruska, K. P., and Tricas, T. C. (1998). Morphology of the mechanosensory lateral line system in the Atlantic stingray, *Dasyatis sabina*: the mechanotactile hypothesis. *Journal of Morphology* **238**, 1–22. doi:10.1002/(SICI)1097-4687(199810)238:1<1::AID-JMOR1>3.0.CO;2-D

McGowan, D. W., and Kajiura, S. M. (2009). Electroreception in the euryhaline stingray, *Dasyatis sabina*. *The Journal of Experimental Biology* **212**, 1544–1552. doi:10.1242/JEB.025247

Peters, R. C., and Bretschneider, F. (1972). Electric phemonena in the habitat of the catfish *Ictalurus nebulosus*. *Journal of Comparative Physiology* **81**, 345–362. doi:10.1007/BF00697755

Robertson, J. D. (1953). Further studies on ionic regulation in marine invertebrates. *The Journal of Experimental Biology* **30**, 277–296.

Shibuya, A., Zuanon, J., de Araújo, M. L. G., and Tanaka, S. (2010). Morphology of lateral line canals in Neotropical freshwater stingrays (Chondrichthyes: Potamotrygonidae) from Negro River, Brazilian Amazon. *Neotropical Ichthyology* **8**, 867–876. doi:10.1590/S1679-62252010000400017

Stein, B. E., and Meredith, M. A. (1993). 'The Merging of the Senses.' (Ed M. S. Gazzaniga.) (The MIT Press: Cambridge, MA.)

Szabo, T., Kalmijn, A. J., Enger, P. S., and Bullock, T. H. (1972). Microampullary organs and a submandibular sense organ in the fresh water ray, *Potamotrygon*. *Journal of Comparative Physiology* **79**, 15–27. doi:10.1007/BF00693615

Szamier, R. B., and Bennett, M. L. V. (1980). Ampullary electroreceptors in the fresh water ray, *Potamotrygon*. *Journal of Comparative Physiology. A. Neuroethology, Sensory, Neural, and Behavioral Physiology* **138**, 225–230. doi:10.1007/BF00657040

Taylor, N. G., Manger, P. R., Pettigrew, J. D., and Hall, L. S. (1992). Electromyogenic potentials of a variety of platypus prey items: an amplitude and frequency analysis. In 'Platypus and Echindas'. (Ed. M. L. Augee.) pp. 216–224. (The Royal Zoological Society of NSW: Sydney.)

Tricas, T. C., and New, J. G. (1997). Sensitivity and response dynamics of elasmobranch electrosensory primary afferent neurons to near threshold fields. *Journal of Comparative Physiology. A. Neuroethology, Sensory, Neural, and Behavioral Physiology* **182**, 89–101. doi:10.1007/S003590050161

Tricas, T. C., Michael, S. W., and Sisneros, J. A. (1995). Electrosensory optimization to conspecific phasic signals for mating. *Neuroscience Letters* **202**, 129–132. doi:10.1016/0304-3940(95)12230-3

Wood, C. M., Matsuo, A. Y. O., Gonzalez, R. J., Wilson, R. W., Patrick, M. L., and Val, A. L. (2002). Mechanisms of ion transport in *Potamotrygon*, a stenohaline freshwater elasmobranch native to the ion-poor blackwaters of the Rio Negro. *The Journal of Experimental Biology* **205**, 3039–3054.

Wueringer, B. E., Squire, L. J., Kajiura, S. M., Tibbetts, I. R., Hart, N. S., and Collin, S. P. (2012). Electric field detection in sawfish and shovelnose rays. *PLoS ONE* **7**, e41605. doi:10.1371/JOURNAL.PONE.0041605

Marine and Freshwater Research 2015, **66**, 1027–1036
http://dx.doi.org/10.1071/MF14354_AC

Supplementary material

Electroreception in the obligate freshwater stingray, *Potamotrygon motoro*

Lindsay L. Harris[A], *Christine N. Bedore*[B] *and Stephen M. Kajiura*[A,C]

[A]Department of Biological Sciences, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33431, USA.

[B]Biology Department, Duke University, Durham, NC 27708, USA.

[C]Corresponding author. Email: kajiura@fau.edu

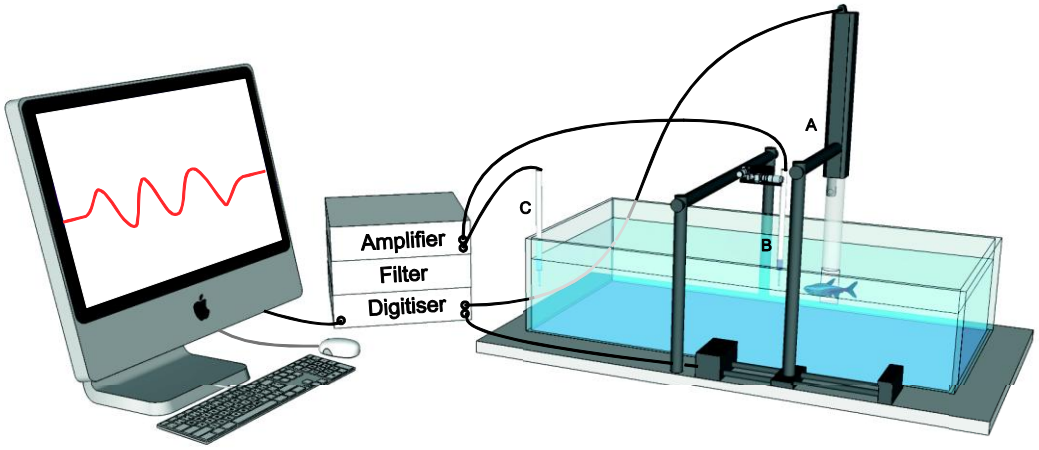

Fig. S1. Electrophysiological apparatus used to measure voltage produced by a living organism. A representative prey item was secured to a submerged stage on the arm of a linear translation system (A). The tip of a recording electrode (B) was placed at various locations along the body, and the voltage from the recording and reference (C) electrodes was differentially amplified, filtered, digitised, and visualised on a computer. For the distance trials, the fish was moved in automated 1-cm increments away from the recording electrode using the linear translation system.

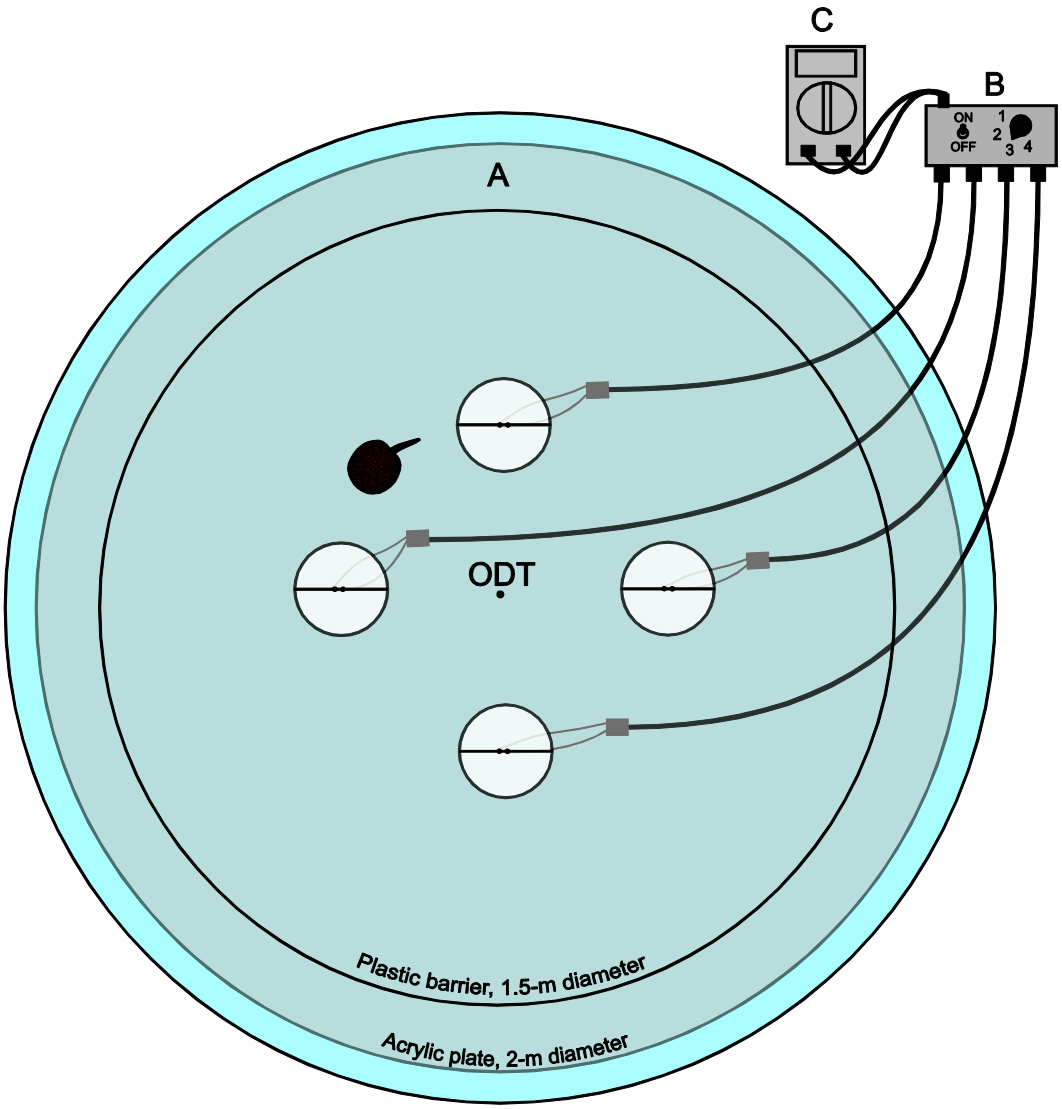

Fig. S2. Simplified top-down view of experimental apparatus for behavioural sensitivity trials. An acrylic plate 2 m in diameter (A) rested on the bottom of the experimental tank of equal diameter. Electrodes connected to the centre of each 20-cm-diameter circle on the plate were controlled by a bioelectric field generator (B) that emits an electric current. Current was monitored using a multimeter (C). To encourage prey-searching behaviour, an odour stimulus was introduced into the tank before electrode activation by an odour-delivery tube (ODT) mounted flush to the middle of the plate.