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Behavioral responses of batoid elasmobranchs to prey-simulating electric fields are correlated to peripheral sensory morphology and ecology

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A B S T R A C T

Electrosensory pore number, distribution, and sensitivity to prey-simulating electric fields have been described for many shark species. Electrosensory systems in batoids have received much less attention. Pore number and distribution have yet to be correlated to differences in sensitivity. However, pore number, pore distribution and sensitivity have been linked to behavior, diet, and morphology and follow species-specific trends. We report here that cownose rays have a greater number of pores than the yellow stingray, most of which are concentrated on the anterior ventral surface for both species. However, yellow stingrays have a broader arrangement of pores on both their dorsal and ventral surfaces than the cownose rays. Yellow stingrays demonstrated a median behavioral sensitivity to weak electric fields of $22\,nV\,cm^{-1}$ and are among the most highly sensitive batoids studied to date. Cownose rays are less sensitive than all other elasmobranch species with a median sensitivity of $107\,nV\,cm^{-1}$. As reported in previous studies, a higher pore number did not result in greater sensitivity. Cownose rays are benthopelagic schooling rays and may benefit from reduced sensitivity to bioelectric fields when they are surrounded by the bioelectric fields of conspecifics. Yellow stingrays, on the other hand, are typically solitary and bury in the substrate. A greater number of pores on their dorsal surface might improve detection of predators above them. Also, increased sensitivity and a broader distribution of pores may be beneficial as small prey items move past a buried ray.

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1. Introduction

The ability to detect weak bioelectric fields has evolved independently in several vertebrate lineages (Bullock et al., 1983; Czech-Damal et al., 2012). The most renowned and most sensitive group known to use electroreception is the elasmobranch fishes, the sharks, skates, and rays (Bullock et al., 1983). The elasmobranch electrosensory system is composed of receptor cells that line the lumen of a bulbous ampulla, which leads to a canal filled with a conductive glycoprotein gel that terminates as a pore on the surface of the skin (Hueter et al., 2004). These pores are the interface between the seawater environment and the internal environment of the animal. The receptors detect a voltage gradient between the pore and the reference potential at the receptor cell. The pores are distributed in species-specific arrangements on the head of sharks

and on the dorsal and ventral body surfaces of skates and rays (Raschi, 1986; Kajiura et al., 2010).

Pore distribution has been correlated with habitat, diet, morphology, and lifestyle (Raschi, 1986; Raschi and Mackanos, 1987; Kajiura, 2001; Jordan, 2008; Wueringer and Tibbets, 2008; Kajiura et al., 2010; Wueringer et al., 2011, 2012), but neither pore distribution nor pore number have been correlated to sensitivity (Kajiura, 2001; Kajiura and Holland, 2002; Jordan et al., 2009). Nonetheless, pore number and distribution may still contribute to behavioral responses to bioelectric fields, perhaps by providing an increase in spatial resolution. For example, Raschi (1986) correlated high pore densities in skates with diets composed of immobile infaunal invertebrates and hypothesized that high pore densities increase spatial resolution during prey localization. Additionally, broad distributions of pores across dorsal and ventral surfaces of skates were correlated with generalized diets of mobile infaunal and epibenthic invertebrates, as well as with mobile pelagic prey like squid and teleosts (Raschi, 1986). These broad distributions are thought to expand the electrosensory search area so that prey farther from the mouth can be more easily detected.

The batoid order Myliobatiformes exhibits a wide range of morphologies, lifestyles, habitats, and diets (Fig. 1), which may correlate

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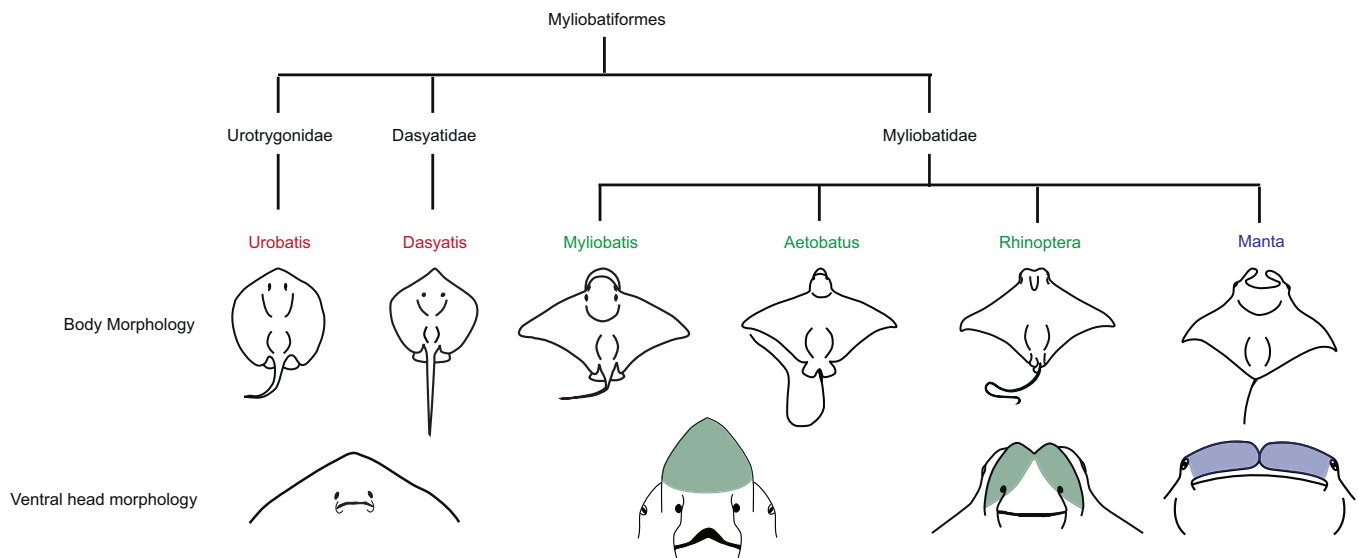
Fig. 1. Morphological variation in head shape within the order Myliobatiformes. An increasing complexity in cephalization and cephalic lobe structure is evident in the phylogeny of the group. *Urobatis* and *Dasyatis* are basal, benthic rays that lack cephalic specialization. *Myliobatis* and *Aetobatus* are benthopelagic rays with a single lobed, enlarged rostrum. *Rhinoptera* is a derived benthopelagic ray that has a bilobed head shape with paired extendable lobes on the ventral surface of the head. *Manta* also has paired cephalic lobes and is fully pelagic. Whereas *Rhinoptera* cephalic lobes possess electrosensory pores, cephalic fins in *Manta* do not. Shading represents the presence of cephalic specialization or lobes.

with pore distribution and the capability to detect and localize prey electrically. Yellow stingrays (*Urobatis jamaicensis*) are basal myliobatids with a strictly benthic lifestyle and generalized diet (Yáñez-Arancibia and Amézcua-Linares, 1979) and morphology. Cownose rays (*Rhinoptera bonasus*) are among the most highly derived myliobatid rays and have adopted a benthopelagic lifestyle and specialized diet composed primarily of weakly electric immobile or slow-moving benthic prey (Smith and Merriner, 1985; Collins et al., 2007; Ajemian and Powers, 2012; Bedore and Kajiura, 2013). Cownose rays possess a head morphology with paired extensions from the ventral surface (cephalic lobes) that are unique to the genus (Fig. 1). The cephalic lobes are used to excavate prey from the substrate and are thought to contain a high density of electrosensory pores (Chu and Wen, 1979). However, pore number has not been quantified and a detailed description of pore distribution in this species is lacking.

Differences in pore number, distribution and density that are correlated to diet, morphology, and lifestyle may be reflected in the behavioral responses of batoids to prey-simulating electric stimuli. In the present study, we quantified pore number, distribution and density, as well as behavioral sensitivity in cownose rays and yellow stingrays. Due to pelagic-associated schooling behavior and preferences by cownose rays for electrically cryptic invertebrate prey, we expected that cownose rays would demonstrate a greater density, but a more narrow distribution of electrosensory pores than yellow stingrays. We expected that cownose rays would be more sensitive to prey-simulating electric stimuli than yellow stingrays.

2. Materials and methods

2.1. Electrosensory pore number and distribution

To determine the influence of electrosensory pore distribution and density on behavior, the number of pores was quantified from five cownose rays, *Rhinoptera bonasus* (disc width (DW) 36–57 cm; 3 female, 2 male), and five yellow stingrays, *Urobatis jamaicensis* (DW 16–21 cm; 3 female, 2 male), obtained from incidental mortalities in other studies or from the Florida Museum of Natural History Ichthyology Collection. Individual pores were counted

under a magnifying lens and each pore was marked to prevent recounting. To minimize bias of a single observer, the left and right sides of each specimen were counted by different observers. The two counts were summed to provide dorsal, ventral, and total pore number.

High-resolution digital photographs of one specimen of each species were used to construct a digital representation of the electrosensory pore distribution on the bodies of the cownose ray and yellow stingray. The digital pore map was then used to quantify dorsal and ventral pore coverage areas and the density of pores within the respective dorsal and ventral pore fields. The pore fields and body surface areas were calculated by placing landmarks on the pore map to accurately represent the outlines of the respective surface areas (adapted from Jordan, 2008). Body surface landmarks were: 1. most anterior point on the rostrum (yellow stingrays) or cephalic lobe (cownose rays), 2. most lateral point of the pectoral fin margin, 3. pectoral–pelvic fin intersection, and 4. most posterior point of the cloaca. Dorsal pore field landmarks were: 1. most anterior pore on the rostrum or cephalic lobe, 2. most lateral pore on the disc–pectoral fin margin, 3. and most posterior pore on the head. Ventral pore field landmarks were: 1. most anterior pore on the rostrum or cephalic lobe, 2. most lateral pore on the pectoral fin, 3. most posterior pore on the disc, and 4. most posterior pore on the branchial basket. The surface area (cm^2) delineated by the landmarks for body surface and pore coverage area was quantified using ImageJ (Rasband, 1997–2012). Percent pore coverage was taken as the proportion of the body surface that contained the pore field. Density was calculated as total number of pores contained within the pore coverage area (pores cm^{-2}). To minimize the effects of differences in density due to body size and shape, the pore map of both species was scaled to the same disc length (20 cm).

2.2. Behavioral sensitivity

2.2.1. Animal collection and maintenance

Behavioral electrosensitivity for 12 juvenile cownose rays (DW 35–53 cm; 6 female, 6 male) and 8 adult yellow stingrays (DW 17–20 cm; 6 female, 2 male) was quantified. Cownose rays were collected by gill net and cast net from Sarasota Bay, Florida, USA.

Animals were maintained for 3–7 nights in a holding tank at Mote Marine Laboratory in Sarasota prior to their transport to the Florida Atlantic University (FAU) Marine Science Laboratory at Gumbo Limbo (GL) Environmental Complex in Boca Raton. Yellow stingrays were collected by hand-net from patch reefs near Long Key, Florida. Rays were held in holding tanks at Keys Marine Laboratory in Layton for 1–3 nights before transport to the FAU GL facility.

All rays were held in large indoor tanks with flow-through seawater at temperatures of 25–29 °C and salinity of 35 ppt prior to and during behavior trials. Cownose rays were held in a 4.9 m diameter round tank and yellow stingrays in a 1.2 m × 2.4 m × 1.2 m rectangular tank. Rays were fed to satiation daily for a minimum of one month before experiments. All rays were individually tagged with colored plastic tags for identification during the experimental period. All procedures with live animals were approved by the Florida Atlantic University Institutional Animal Care and Use Committee (#A08-34).

2.2.2. Experimental apparatus and protocol

A behavioral assay was employed to quantify the sensitivity of cownose rays and yellow stingrays to prey-simulating electric fields. Cownose rays were tested within the 4.9 m holding tank. A barrier constructed from PVC pipe and plastic mesh was used to isolate the experimental animals from the other rays in the tank. Yellow stingrays were tested in a rectangular tank, identical in its dimensions to that of the holding tank. Single cownose rays were unresponsive to electric stimuli, so two rays were tested simultaneously in all trials for both species.

A 1 m × 1 m acrylic plate was placed on the bottom of the experimental arena in each tank. For cownose rays, graded acrylic ramps were secured to the acrylic plate to provide a smooth transition from the tank bottom onto the acrylic plate. For yellow stingrays, the acrylic plate was placed on the sand and the margins buried to blend the plate with the substrate. Four electric dipoles, each with a separation distance of 1 cm, were equally spaced on the plate. Seawater-filled polyethylene aquarium tubing was fitted on the underside of the plate at each dipole to provide a salt bridge. The tubing was connected to a pair of gold-plated stainless steel electrodes joined to an underwater cable (Impulse Enterprise, San Diego, CA, USA). The four electrodes were connected to an electric stimulator (cf. Kajiura and Holland, 2002) powered by a 9 V battery with a multimeter connected in series to monitor the applied current. A 20 cm diameter reference circle surrounded each dipole to provide a calibration measurement for subsequent video analysis (Fig. 2).

Food was withheld prior to experimentation to ensure rays were motivated to behaviorally respond to prey-simulating stimuli. Due to differences in metabolic demand between the species, cownose rays were fasted for 24 h while yellow stingrays were fasted for 48 h. At the beginning of a trial, we placed two rays into the experimental arena or tank and allowed them to acclimate to the dipole array for a period of 30–60 min. Upon acclimation, a food odor (squid or scallop rinse) was delivered to the tank through an odor delivery tube mounted flush to the center of the array. These odors initiated prey-searching behavior. When the ray began to forage near the electrode array, the odor delivery was stopped and a 12 μA prey-simulating electric stimulus (Bedore and Kajiura, 2013) was applied randomly to one of the four electrode pairs, with the three inactive dipoles acting as controls. Once a ray bit at an active dipole, the electrode pair was immediately switched off and another dipole was randomly activated. Trials lasted a maximum of 1 h and each ray was tested a maximum of three times. Responses were recorded with a Sony HDR-CX260 digital video camera (30 frames s⁻¹; Sony Corp., Tokyo, Japan) mounted above the center of the array, 1.5 m above the water surface.
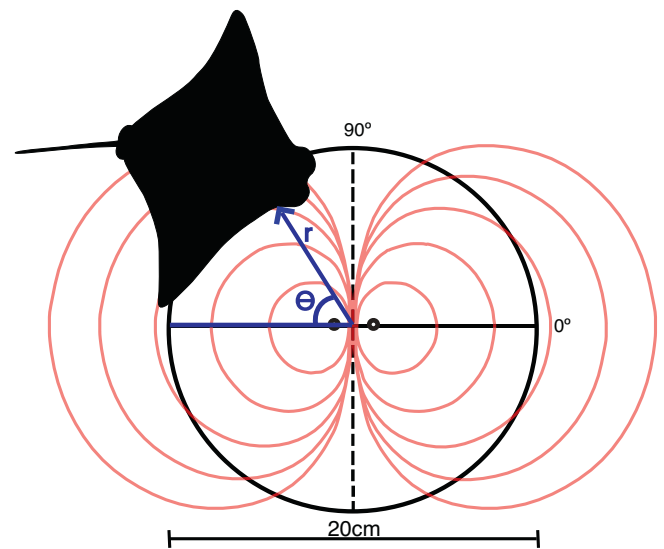

Fig. 2. Orientation measurements used to calculate sensitivity to dipole electric fields. Orientation distance (r) and angle with respect to the dipole axis (θ) were used to calculate the voltage gradient at the point where a ray initiated a turn toward the dipole source. The electric field intensity decreases with increasing distance from the center of the dipole. The electric field also decreases as a cosine function and is strongest along the dipole axis (0°) while no detectable field is produced perpendicular to the axis (90°). A 20 cm diameter circle on the electrode array provided a calibration to quantify orientation distance.

2.2.3. Video analysis

Video footage was imported into a computer, edited with iMovie v 7.1.4 (Apple Inc., Cupertino, CA, USA) and analyzed using ImageJ (Rasband, 1997–2012). Bite responses that included a change in swimming trajectory as the dipole was approached (orientation) were used to calculate the strength of the electric field at the point of orientation as a measure of behavioral sensitivity to prey-simulating electric fields. The frame in which an orientation was initiated was exported as a still image and the distance (r) and angle (θ) to the dipole axis at the point of orientation were measured (Fig. 2). To ensure rays did not influence responses of one another, frames in which both rays were present were excluded from analysis. The electric field strength (E) at which a ray initiated a response to the electric stimulus (sensitivity) was calculated for all orientation responses using the following ideal dipole equation (Kalmijn, 1982; Kajiura and Holland, 2002):

$$E = \frac{\rho I d}{\pi r^3} \cos \theta \tag{1}$$

where ρ = resistivity of seawater (18.0–19.2 mΩ cm), I = applied current (12.0 μA), d = dipole separation distance (1 cm), r = orientation distance (cm), and θ = orientation angle (with respect to the dipole axis; degrees) (Fig. 2). Orientation distance was measured to the nearest hyoid ampullary cluster (following McGowan and Kajiura, 2009 and Jordan et al., 2009), which is thought to be the primary group involved in the initial stages of electric field detection (Camperi et al., 2007; Kempster et al., 2013).

2.3. Statistical analyses

2.3.1. Electrosensory pore number

ANOVA was used to test for significant differences in dorsal, ventral, and total pore counts between species and paired t-tests to determine if there were significant differences between dorsal and ventral pore counts within a species. Regression analysis was used to determine if pore number varied significantly with disc width.

Table 1
Electrosensory pore number, density, and pore coverage area in two species of batoids.

	Cownose ray (*Rhinoptera bonasus*)		Yellow stingray (*Urobatis jamaicensis*)	
	Dorsal	Ventral	Dorsal	Ventral
Pore number (±SD)	134.4 ± 8.1	1096.4 ± 107.5	187.4 ± 14.3	627.2 ± 25.9
Coverage area (%)	11.7	19.7	23.6	46.7
Density (pores cm^{-2})	1.7	8.3	2.5	4.2

2.3.2. Behavioral sensitivity

Responses > 1 μV cm^{-1} were omitted from analysis (10% for cownose rays and 0% for yellow stingrays) because they occurred too close to the dipole to be measured accurately. Non-parametric analyses were used to determine if there were significant differences between species in orientation distance (cm) and sensitivity (nV cm^{-1}). Generalized linear models (GLM) were used to determine if there was an effect of sex or disc width on orientation distance and sensitivity within each species. Orientation distance and sensitivity data were log-transformed to achieve normality. Because behavioral responses are typically right-tailed, median values were used to compare sensitivity and detection distance between the two species (Sokal and Rohlf, 1994; Kajiura and Holland, 2002; Kajiura, 2003; Jordan et al., 2009; McGowan and Kajiura, 2009). All statistical analyses were performed using JMP statistical software (v. 9.0.2; SAS Institute, Cary, NC, USA) and $\alpha = 0.05$.

3. Results

3.1. Electrosensory pore number and distribution

The number of electrosensory pores for each species is reported in Table 1. Cownose rays had significantly fewer dorsal pores than the yellow stingray (ANOVA: $F_{1,13} = 26.29$, $P < 0.001$), but a significantly greater number of ventral and total pores ($F_{1,13} = 185.29$, $P < 0.001$ and $F_{1,13} = 118.79$, $P < 0.001$, respectively; Fig. 3). Dorsal pore number was significantly lower than ventral pore number in both species (paired *t*-test: cownose ray: $t_4 = 21.04$, $P < 0.001$; yellow stingray: $t_4 = 50.34$, $P < 0.001$). Pore number did not vary with disc width for either species (regression analysis; cownose ray: $R^2 = 0.48$, $F_{1,3} = 2.75$, $P = 0.20$; yellow stingray: $R^2 = 0.21$, $F_{1,3} = 0.82$, $P = 0.43$).

Cownose rays had smaller dorsal and ventral pore coverage areas than yellow stingrays (Fig. 3 and Table 1). Pores on the dorsal surface of the cownose ray were limited to the medial anterior portion of the body and extended just posterior and lateral to the branchial basket. Pores on the dorsal surface of the yellow stingray extended from the most anterior margin of the body to the most posterior margin of the disc. Pores extended laterally to approximately halfway between the midline and lateral margin of both pectoral fins. Pores on the ventral surface of both species occurred primarily on the anterior portion of the body, with most pores located anterior to the fifth gill slit and a high concentration of pores around the mouth. Ventral pores of yellow stingrays, however, extended along the lateral margin of the disk and had a lower density within the ventral pore field than those of cownose rays (Table 1). Ventral pores of the cownose ray were more concentrated anteriorly than those of yellow stingrays, with the greatest concentration of pores around the mouth and on the margins of the cephalic lobes.

3.2. Behavioral sensitivity

Cownose rays and yellow stingrays demonstrated 625 and 295 bite responses, respectively, to prey-simulating dipole electric fields (Table 2). Of those, cownose rays demonstrated 347

Table 2
Summary of behavioral responses of two species of batoids to prey-simulating electric fields. The best response and maximum orientation distance were calculated from an individual of each species. Median sensitivity and distance were calculated from all responses < 1000 nV cm^{-1} for each species. % inaccurate bites were calculated as a percentage of all bites.

Response	Cownose ray	Yellow stingray
Total number of bites	625	295
Bites with orientation	313	141
Orientations per individual (mean ± SD)	28.9 ± 12.2	18.3 ± 6.3
% orientations < 1000 nV cm^{-1}	90.2	100.0
% orientations < 100 nV cm^{-1}	43.5	85.8
Median detection distance (cm)	7.5	11.2
Median sensitivity (nV cm^{-1})	107	22
Maximum orientation distance	26.8	30.8
Best response (nV cm^{-1})	0.31	0.23
% inaccurate bites	10.9	38.0

orientations to the dipole and yellow stingrays demonstrated 141 orientations. Cownose rays initiated orientations to dipoles from significantly closer distances and hence greater electric field strengths than yellow stingrays (Kruskal–Wallis: $H_1 = 63.73$, $P < 0.001$ and $H_1 = 65.09$, $P < 0.001$, respectively). For cownose rays the median orientation distance was 7.5 cm and the maximum orientation distance was 26.8 cm (Fig. 4). These values yielded a median sensitivity of 106.5 nV cm^{-1} for cownose rays and a minimum electric field detected, or best response, of 0.3 nV cm^{-1}. For yellow stingrays the median orientation distance was 11.1 cm and the maximum orientation distance was 30.8 cm. These values yielded a median sensitivity of 22.6 nV cm^{-1} and a minimum electric field detected of 0.2 nV cm^{-1}. Cownose rays demonstrated 43.5% of their responses to electric fields < 100 nV cm^{-1}, whereas yellow stingrays demonstrated 85.8% of responses < 100 nV cm^{-1}. Sex and disc width did not have an effect on orientation distance (GLM: cownose rays: $F_{2,310} = 0.33$, $P = 0.72$; yellow stingrays: $F_{2,138} = 1.60$, $P = 0.21$) or sensitivity (cownose rays: $F_{2,310} = 0.24$, $P = 0.79$; yellow stingrays: $F_{2,138} = 0.36$, $P = 0.70$). Both rays failed to respond to the active dipole a small number of times. Neither species bit at an inactive (control) dipole. Both rays also occasionally failed to bite at the target and instead bit elsewhere near the target. 68 of the total number of bites (10.9%) for cownose rays were ≥1 cm outside of the target, whereas 112 bites (38.0% of the total number of bites) were inaccurate for yellow stingrays. Inaccurate cownose bites were 4.9 ± 2.2 cm (mean ± SD) from the dipole center and inaccurate yellow stingray bites were 3.4 ± 1.6 cm from the dipole center.

4. Discussion

The peripheral morphology and behavioral sensitivity of the electrosensory system in two batoids were quantified to compare potential differences in sensitivity that may result from morphology, diet, or behavior. Whereas the hypothesis that the cownose ray will possess a greater number, but more limited distribution of pores, was supported, the hypothesis that the cownose ray will demonstrate greater sensitivity to prey-simulating electric fields than the yellow stingray was rejected.
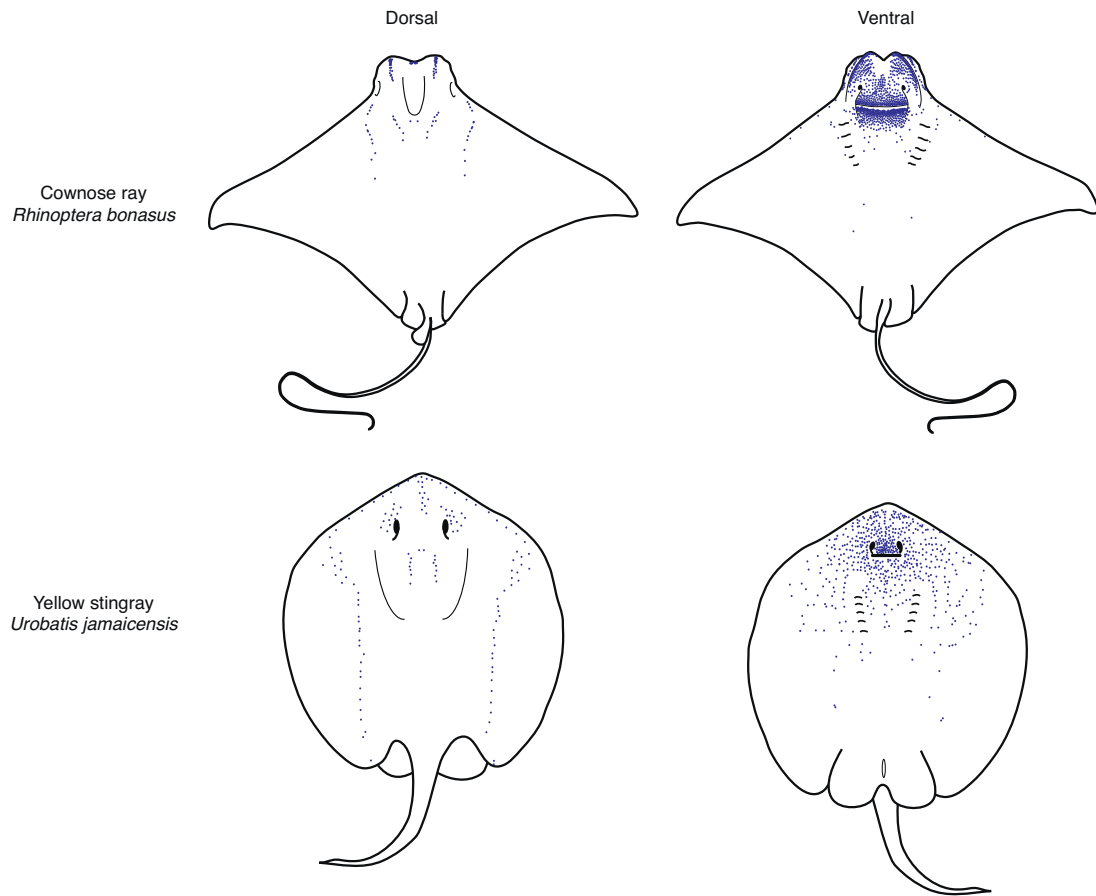
Dorsal   Ventral

Cownose ray
Rhinoptera bonasus

Yellow stingray
Urobatis jamaicensis

Fig. 3. Electrosensory pore number and distribution of the cownose ray, *Rhinoptera bonasus*, and the yellow stingray, *Urobatis jamaicensis*. Both species had fewer dorsal pores than ventral pores and these pores were more limited in distribution on the cownose ray where the pores occurred only on the head and near the gills. Ventral pore distribution was more widespread on the yellow stingray and nearly extended to the lateral disc margins. Ventral pores on the cownose ray were again limited to the head. The highest concentrations of pores in both species was around the mouth; however, pores were more highly concentrated around the mouth and on the cephalic lobes on the cownose ray than on the yellow stingray.

4.1. Electrosensory pore number and distribution

The number of electrosensory pores for the two batoids in this study, the cownose ray, *R. bonasus*, and the yellow stingray, *U. jamaicensis*, were within the ranges reported for other batoids (Raschi, 1986; Jordan, 2008; Wueringer and Tibbets, 2008; Wueringer et al., 2011; Kempster et al., 2012). As predicted, the yellow stingray had a significantly greater number of dorsal pores than the cownose ray; the yellow stingray is strictly benthic and these pores are likely an advantage to benthic rays for electrical detection of signals emitted from predators that swim overhead. In contrast, the cownose ray is benthopelagic and is typically higher in the water column than most electrical signals the rays would encounter.

Both species feed on benthic prey and, therefore, both benefit from a large number of ventral pores. Although both species possess more pores on the ventral surface than the dorsal surface, the cownose ray had a significantly greater number of pores on the ventral surface than the yellow stingray. The distribution of both dorsal and ventral pores was more widespread for the yellow stingray. Pores on the cownose ray were limited to the head and anterior wing margin, whereas pores on the yellow stingray extended from anterior to posterior and out to the lateral disc margins. On the ventral surface of the cownose ray, pores were highly concentrated around the mouth and anterior margins of the head. The limited distribution and high density of pores around the mouth is thought to increase resolution for detection of immobile buried and cryptic prey items (Raschi, 1986), like bivalve molluscs and

echinoderms, which are major prey types of cownose rays (Smith and Merriner, 1985; Collins et al., 2007; Ajemian and Powers, 2012). The more widespread distribution in the yellow stingray is likely to increase the electroreceptive surface area on the body, which confers a greater search area to detect their mobile infaunal prey, like polychaetes and small crustaceans (Yáñez-Arancibia and Amézcua-Linares, 1979; Raschi, 1986).

Pore distribution can also be influenced by other factors, including swimming style and body morphology. Cownose rays swim with large oscillatory flapping movements of the wings (Rosenberger, 2001; Schaefer and Summers, 2005), and pores located at the wing tips would experience significant electrical noise due to their movement through the seawater. This likely constrains pore distribution to the immobile portions of the body with lateral extension of pores terminating medial to the axis of bending in the pectoral fin (Schaefer and Summers, 2005). Conversely, the yellow stingrays swim by small undulations of the lateral fin margin (Rosenberger, 2001; Schaefer and Summers, 2005), so pores can be positioned closer to the lateral disc margin without compromising the electrical signal that they receive.

The body shape of the yellow stingray is typical of that of basal Myliobatiformes (Fig. 1) and the pore distribution is similar to that of the closely related round stingray and Atlantic stingray (Jordan, 2008; McEachran and Aschliman, 2004). The cownose ray is similar in body shape to the more derived Myliobatiformes (Fig. 1) with laterally expanded pectoral fins and increasing degrees of cephalization. For example, the eagle rays and bat rays have a single, enlarged rostrum (Karl and Obrebski, 1976; Sasko et al., 2006;
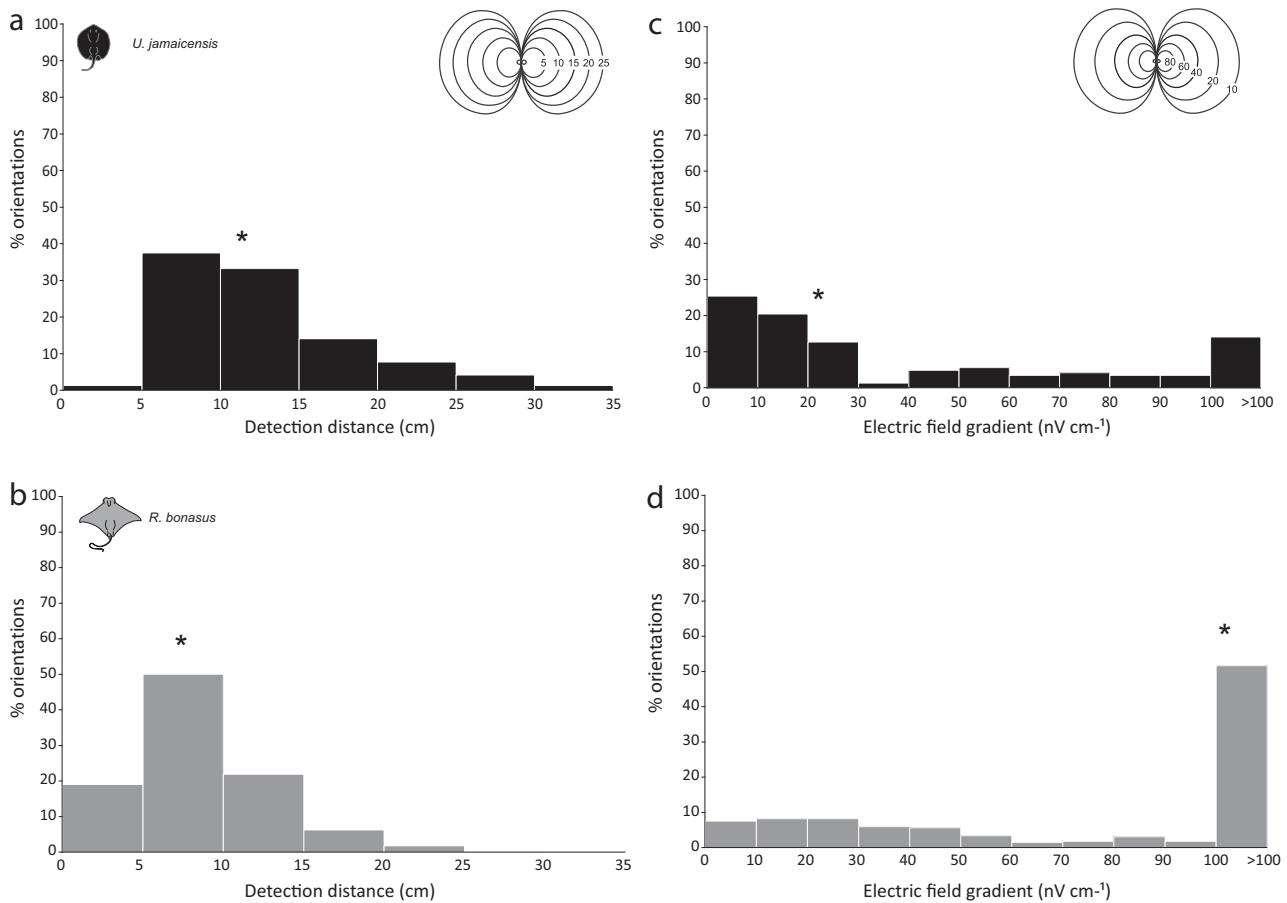
Fig. 4. Percentage of orientations by sensitivity (voltage gradient) and detection distance to prey-simulating electric fields. Yellow stingrays oriented to weaker electric fields (a) and at greater distances (c) than cownose rays (b and d, respectively). Orientations to weaker electric fields and at greater detection distances indicate greater sensitivity to bioelectric fields because the electric field decreases in intensity with distance from the source (insets in (a) and (c)). 43.5% of cownose ray responses were <100 nV cm^{-1}, while 85.8% of yellow stingray responses were <100 nV cm^{-1}. * indicates median sensitivity or detection distance.

Schluessel et al., 2010; Ajemian et al., 2012). Cephalization is more pronounced in the most derived Myliobatiformes, the cownose rays, mantas, and mobulas, which have paired extensions of the anterior pectoral fins, called cephalic lobes, on the anterior margin of the head (González-Isáis and Domínguez, 2004; McEachran and Aschliman, 2004; Sasko et al., 2006). In all species with some degree of cephalization, the rostrum or cephalic lobes are involved in feeding and can function in prey manipulation and excavation, as well as detection and localization (Karl and Obrebski, 1976; González-Isáis and Domínguez, 2004; Sasko et al., 2006; Schluessel et al., 2010; Adnet et al., 2012; Ajemian et al., 2012). The cephalic lobes of cownose rays are more flexible than those of other Myliobatiformes (González-Isáis and Domínguez, 2004; Sasko et al., 2006) and are inundated with electrosensory pores, in contrast to mobulas and mantas, which lack electrosensory structures on the cephalic lobes (Chu and Wen, 1979). Electrosensory pores on the cephalic lobes of cownose rays likely contribute to high resolution during feeding when the lobes are extended and a ray is searching for prey along the substrate. Although it has been suggested that high pore densities result in increased resolution (Raschi, 1986), this hypothesis has yet to be empirically tested.

4.2. Behavioral sensitivity

Both yellow stingrays and cownose rays detected electric fields < 0.5 nV cm^{-1}, similar to other batoid and shark species studied with the same technique (Table 3). However, their median sensitivities differed, with the yellow stingray demonstrating much greater sensitivity than the cownose ray. The yellow stingray initiated 85.8% of responses to stimuli at < 100 nV cm^{-1} whereas the cownose ray initiated only half that number (43.5%) of responses to voltage gradients < 100 nV cm^{-1}. Although the electric stimuli were identical for both species, differences in physiology and behavior between the two rays required variations in the experimental design that could have affected responses.

Cownose rays are physically larger and are active swimmers, so they required a greater absolute search area in behavior trials. Although a larger search area alone should decrease responsiveness in cownose rays by decreasing the chances of interacting with the dipole, their activity level offset the differences in experimental arena size. Cownose rays spent considerably more time searching for prey, which resulted in an increased number of responses by each ray compared to yellow stingrays. The lower sensitivity in the cownose ray may have been an artifact of decreased motivational state when compared to the yellow stingrays, although this is unlikely. Yellow stingrays were fasted 48 h before each trial and cownose rays 24 h before each trial. The metabolic physiology of these rays necessitated these differences in fasting periods; yellow stingrays are similar to other benthic batoids that rest buried in the substrate for most of the day and, therefore, have a slow metabolism (Di Santo and Bennett, 2011). Cownose rays exhibit a two-fold increase in metabolic rate when kept at the same temperature as a benthic batoid (Neer et al., 2006; Di Santo and Bennett, 2011), so fasting for 48 h would have had detrimental effects on the health of cownose rays. Differences in dietary composition between the two species may have contributed to differences in

Table 3
Behavioral electrosensitivity of batoid elasmobranchs.

Species	Common name	Minimum (nV cm^{-1})	Median (nV cm^{-1})	Lifestyle	Reference
Rhinoptera bonasus	Cownose ray	0.3	107	Benthopelagic	Present study
Urobatis jamaicensis	Yellow stingray	0.2	22	Benthic	Present study
Myliobatis californica	Bat ray	0.1	48	Benthopelagic	Jordan et al. (2009)
Urobatis halleri	Round stingray	0.3	29	Benthic	Jordan et al. (2009)
Pteroplatytrygon violacea	Pelagic stingray	0.3	40	Pelagic	Jordan et al. (2009)
Dasyatis sabina	Atlantic stingray	0.6	5	Benthic	McGowan and Kajiura (2009)
Pristis microdon	Freshwater sawfish	–[a]	13	Benthic	Wueringer et al. (2012)
Glaucostegus typus	Giant shovelnose ray	–[a]	25	Benthic	Wueringer et al. (2012)
Aptychotrema rostrata	Eastern shovelnose ray	–[a]	80	Benthic	Wueringer et al. (2012)

[a] Not reported.

responsiveness, with the prey-simulating stimuli being more attractive to foraging cownose rays than yellow stingrays. Although the diets of yellow stingrays have considerable overlap with those of cownose rays in southwest Florida (Yáñez-Arancibia and Amézcua-Linares, 1979; Collins et al., 2007), cownose ray diets typically contain a greater proportion of stationary or slow-moving invertebrates than those of yellow stingrays. Our prey-simulating stimulus was strictly direct current, which is more similar to a bivalve with a constant flow of water through the gills than to other invertebrates which have an additional, modulated component associated with rhythmic gill movements (Kalmijn, 1974; Bauer, 1999; Bedore and Kajiura, 2013). Both species readily searched for food when the prey odor was introduced, and both species vigorously bit at the dipole and consumed food during trials, so the stimulus was likely appropriate for replicating a range of invertebrate prey items for both species.

Life history characteristics and feeding behavior are more likely to describe the differences in sensitivity between the species than variations in experimental design. The diet of cownose rays and yellow stingrays may contribute to differences in feeding behavior between the two species. The combined electric and mechanical (lateral line) stimuli produced by mobile prey items provide yellow stingrays with the required information to localize a prey item in the absence of visual cues during prey striking behavior with their ventrally positioned mouth (Maruska, 2001; Gardiner et al., 2012). A large part of the juvenile cownose ray diet consists of weakly electric (Bedore and Kajiura, 2013) immobile or slow-moving infauna (Smith and Merriner, 1985; Collins et al., 2007; Ajemian and Powers, 2012). The high density of pores surrounding the mouth and on the cephalic lobes may increase resolution and accuracy in the localization of a stationary, buried prey item in the absence of lateral line and visual stimuli (Raschi, 1986; Jordan et al., 2009). During behavior trials, yellow stingrays failed to accurately locate the center of the prey-simulating dipole in 38% of bite responses, whereas cownose rays failed to localize the target only 10% of the time, thus supporting the hypothesis that increased pore density leads to increased spatial resolution.

The schooling behavior of cownose rays may also affect behavioral sensitivity. Schooling rays are perpetually surrounded by conspecifics, each with their own bioelectric field. A large proportion of dorsal pores aids in the detection of conspecifics by benthic batoids (Tricas et al., 1995; Sisneros et al., 1998; Sisneros and Tricas, 2002) and could benefit cownose rays in maintaining position within a school. However, rays may experience overstimulation of the electrosensory system while schooling due to exposure to the additive voltage component of electric fields by schoolmates, as well as from asynchronous wing movements of different frequencies and periodicity by each neighbor (C.N.B., pers. observ.). Also, the electrical noise created by schooling conspecifics likely exceeds the electric field strengths of their prey, which can be as low as 10–30 µV (Kalmijn, 1974; Haine et al., 2001; Bedore and Kajiura, 2013). The small number of dorsally positioned pores and the

dorsal pore coverage area, in addition to reduced electrosensitivity, may decrease the amount of electrosensory information that reaches the central nervous system for processing. Although pelagic elasmobranchs tend to have smaller cerebellar-like areas for the discrimination of electrosensory information than their benthic counterparts (Kajiura et al., 2010; Yopak et al., 2010; Yopak, 2012), central integration and neuronal convergence from the ampullary neurons to the CNS in pelagic and schooling species need to be considered in future studies to determine differences in information processing that may be related to schooling.

Although cownose rays may experience a decrease in behavioral electrosensitivity, they likely benefit from enhanced visual systems to enable maintenance of position in a school and facilitate prey detection. Cownose rays have laterally positioned eyes, rather than dorsally positioned ones as in most batoids, which affords an expanded vertical field of view, and enables them to visualize objects both above and below their heads (McComb and Kajiura, 2008). The expanded visual field may aid in schooling behaviors and tracking of conspecifics. Although the initial cues used in prey detection by cownose rays are currently unknown, it is interesting to note that rays performed poorly in behavior trials when tested individually and demonstrated a dramatic improvement in prey searching behavior and responsiveness when tested in pairs. Potentially these rays use a cooperative feeding strategy and rely in part on visual conspecific cues to initiate feeding behaviors (Galef and Giraldeau, 2001).

4.3. Behavioral ecology

Electrosensory pore number and distribution are poor predictors of behavioral sensitivity to prey-simulating electric fields. For example, hammerhead sharks benefit from a greater number of pores spread across a larger surface on their laterally expanded cephalofoil, which increases electrosensory search area but not sensitivity (Kajiura, 2001; Kajiura and Holland, 2002). In this study, the cownose ray had a greater number of pores with a higher pore density on the anterior ventral surface of the head, but was less sensitive to prey-simulating electric fields than the yellow stingray. The difference in behavioral sensitivity is likely a reflection of differences in behavior, diet preferences, and body morphology rather than differences in electrosensory pore number. Cownose rays are among the most highly derived batoids and their pore distribution has likely evolved to best fit their benthopelagic schooling behavior and diet preferences. The high density of electrosensory pores surrounding the mouth and on the cephalic lobes may aid in the accurate localization of a weakly electric, stationary prey source, as well as in the discrimination of prey and other bioelectric sources while foraging. Conversely, the yellow stingray was more sensitive to prey-simulating electric fields, but resolution may be sacrificed for a wider distribution of pores that allow a greater electrosensory search area to detect mobile prey. Future studies should empirically test the hypothesis that higher densities

of pores do result in greater resolution. The role of electroreception in schooling elasmobranchs should also be examined in more detail to determine if electrosensory cues are used for maintenance of position within a school. Investigations of multimodal integration in schooling species will also shed light on specific cues used in prey detection while schooling. If sensitivity is decreased because of schooling behavior, cownose rays may rely more heavily on olfactory cues, as well as visual cues from other members within the school to indicate buried invertebrate prey. This study illustrates the complexity of sensory ecology and the fact that species may vary greatly in their sensory capabilities due to a wide range of factors, which should be considered in detail when investigating the significance of sensory system function.

Acknowledgements

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