Available online at www.sciencedirect.com
ScienceDirect
Zoology 112 (2009) 442–450
ZOOLOGY
www.elsevier.de/zool

Electric organ morphology and function in the lesser electric ray, *Narcine brasiliensis*

Laura J. Macesic*, Stephen M. Kajiura

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

Received 24 June 2008; received in revised form 24 February 2009; accepted 24 February 2009

Abstract

The lesser electric ray, *Narcine brasiliensis*, is a small, demersal ray capable of generating electricity through its main and accessory electric organs. Although closely related to the large piscivorous torpedo rays, it differs in size, habitat, and prey. Based on these differences, we hypothesized that the main electric organs are used for predator defense rather than feeding and that the accessory electric organs, specific to this species, are used for intraspecific communication. We found that the mass of the main and accessory electric organs were both significantly smaller in females than in males. Whereas the main electro-somatic index does not change with growth, the accessory electro-somatic index increases, providing support for the accessory electric organs' use in intraspecific communication. We characterized the discharge properties of the main electric organ throughout ontogeny by simulating a predation attempt on the ray. Rays always responded by generating electric organ discharges (EODs) and by flexing the tail dorsoventrally and laterally. The main EOD amplitude, measured directly at the source, increased logarithmically with disc width to a maximum measured amplitude of 56 V. Minimum amplitude was more variable, but followed a positive power relationship with disc width. Neonates produced trains comprised of significantly more EODs than the adults. Over the course of the first set of discharges, all age classes showed a decrease in fundamental frequency and an increase in train duration. In contrast to these defensive responses, the rays did not generate EODs while foraging or feeding on live prey.

Keywords: Torpediniformes; Electro-somatic index; Electric organ discharge; Predator defense; Intraspecific communication

Introduction

Electric organs have evolved independently many times in both freshwater and marine fishes (Bass, 1986; Moller, 1995; Alves-Gomes, 2001; Zupanc and Bullock, 2005). Their uses vary from communication and electrolocation to predatory and defensive functions, depending on the strength and temporal properties of the electric organ discharge (EOD). Within the elasmobranch fishes, only the skates (Rajiformes) and torpedo rays (Torpediniformes) are electrogenic (Nelson, 1994; Moller, 1995; de Carvalho, 1999). Skates possess small, paired electric organs within the tail that emit intermittent weak EODs of variable amplitude (tens of millivolts; Bennett, 1971). These weak EODs are used in intraspecific communication (Bratton and Ayers, 1987). In contrast, the strongly electric torpedo rays generate up to 50 V and 1 kW of electricity from large, paired, kidney-shaped electric organs located within their

*Corresponding author. Tel.: +1 561 297 1040.
E-mail address: lmacesic@fau.edu (L.J. Macesic).

0944-2006/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.zool.2009.02.002

pectoral fins (Bennett et al., 1961). Within the family Torpedinidae, trains of these strong EODs are used for both defense (Belbenoit, 1986; de Carvalho, 1999) and to subdue prey (Coates, 1934; Wilson, 1953; Pfeiffer, 1961; Belbenoit and Bauer, 1972; Bray and Hixon, 1978; Belbenoit, 1986; Lowe et al., 1994). In a predatory context, the piscivorous *Torpedo californica* jumps over its prey, and simultaneously begins emitting several trains of hundreds of EODs. This either stuns or kills the prey, thus allowing for easier prey handling and processing (Bray and Hixon, 1978; Lowe et al., 1994).

The closely related electric ray, *Narcine brasiliensis* (Griffith and Smith, 1834), employs a markedly different foraging strategy than *T. californica*. This benthic electric ray feeds primarily on burrowing polychaetes and small crustaceans (Goitein et al., 1998). To excavate these burrowing organisms, the ray protrudes its jaws into the substrate, generates negative oral pressures, and sucks prey items into its mouth (Dean and Motta, 2004). This electric ray is also considerably smaller than *T. californica*, and therefore possesses smaller electric organs and generates weaker EODs. Although some morphological data and electrical recordings have been obtained from the electric organs of *N. brasiliensis* (Cox and Breder, 1943; Mathewson et al., 1958; Bennett and Grundfest, 1961; Bennett et al., 1961), the behavioral function of the EODs has yet to be determined for this species.

N. brasiliensis is unique among the electrogenic batoids in that it also possesses a pair of smaller, spindle-shaped accessory electric organs (AEOs) located at the posterior margin of each main electric organ (MEO), and oriented obliquely ventral toward the midline (Mathewson et al., 1958) (Fig. 1). Only one other fish, the electric eel *Electrophorus electricus*, also possesses both a strong MEO and a weak AEO (Moller, 1995). The electric eel's MEO is used in predation, whereas the AEO produces monophasic low-voltage signals used in electrolocation (Moller, 1995; Stoddard, 1999). Although the AEOs of *N. brasiliensis* have been mechanically stimulated to generate an EOD (Bennett and Grundfest, 1961), no natural recordings have been made and therefore no behavioral function has been ascribed, although there has been speculation regarding its involvement in social communication (Bennett, 1971; Rudloe, 1989; Rudloe and Rudloe, 1993).

Based on this ray's relatively small size, presumed vulnerability to predators, and diet of infaunal invertebrates, we hypothesized that the MEOs might be used for predator defense rather than feeding. We established three goals which, when combined, would test this hypothesis and, in addition, would generate data to provide support for the AEO's use for intraspecific communication. The goals of this study were to: (1) describe the morphology of the main and AEOs throughout ontogeny; (2) characterize the discharge properties of the MEO throughout ontogeny; and (3) determine the behavioral function of the MEO.

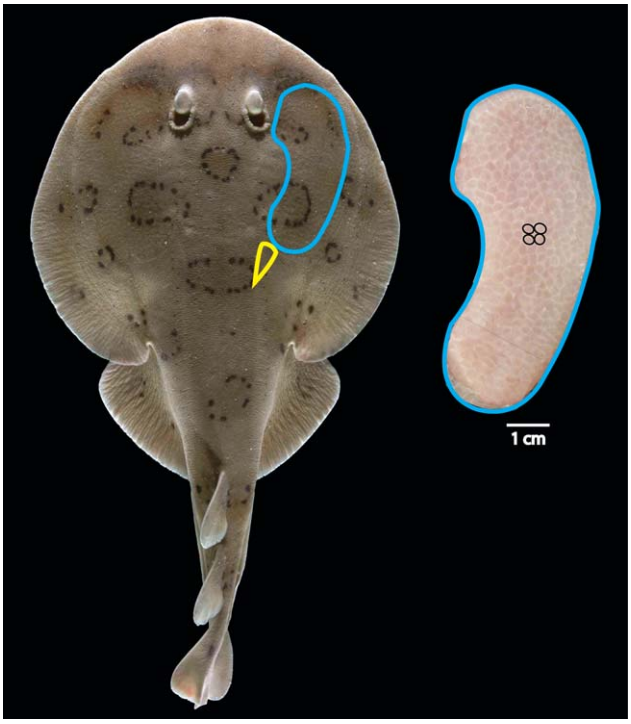

Fig. 1. Dorsal view of *N. brasiliensis* showing the position of the main electric organ in blue, and the position of the accessory electric organ in yellow. Dorsal view of the main electric organ, with several electrocytes outlined in black shown to the right of the ray. Columns of electrocytes were counted from such photographs. Scale bar = 1 cm.

Materials and methods

Morphometrics

Morphometric data were collected from both preserved ($n = 15$) and fresh dead ($n = 15$) lesser electric rays, *N. brasiliensis*. Preserved rays were obtained from the Florida Museum of Natural History, and fresh rays were obtained from shrimp trawlers' bycatch in Panacea, FL, and from our incidental mortalities (resultant of power loss during hurricanes in 2004). For both the preserved and fresh rays, we measured disc width (DW, a measure of body size for batoids), total length (TL), body mass, MEO mass, and AEO mass.

MEO columns of electrocytes were counted using one of two methods. For fresh specimens, the electric organ (Fig. 1) was digitally photographed (Nikon Coolpix 4500; Nikon Corp., Tokyo, Japan) and counts were made on each photograph using the software ImageJ (Rasband, 1997). For preserved specimens, the columns were counted directly on the organ and a dab of nail polish was applied to prevent re-counting. For both fresh and preserved specimens, the number of columns of electrocytes was regressed against DW.

Once the columns of electrocytes were counted, the main electric organ was carefully removed and digitally

photographed with a ruler to provide scale. Surface area of the entire electric organ was measured using ImageJ. We compared the surface area data from fresh and preserved rays using a one-way ANCOVA with DW as the covariate. We also tested for sexual dimorphism of the MEO surface area by using a one-way ANCOVA with DW as the covariate. The same methods and tests were applied to the accessory electric organ.

The organ mass was also compared between fresh and preserved rays for the MEOs and AEOs using one-way ANCOVAs, with DW as the covariate. Masses of each organ throughout ontogeny were then regressed against whole body mass.

From the electric organ mass data, we calculated the proportion of electrogenic mass relative to total body mass, or the electro-somatic index (ESI). This proportion, expressed as a percentage, was compared between preserved and fresh rays for the MEOs and the AEOs using one-way ANCOVAs, with DW as the covariate. Both the MESIs and AESIs were compared between the sexes by using a one-way ANCOVA, with DW as the covariate. We also performed a regression analysis to determine how each ESI changed through ontogeny, again with DW as the sizing variable. ESIs were log transformed to achieve normality and homoscedasticity.

Behavioral function of main electric organ discharges

Collection

A total of 29 *N. brasiliensis* (DW 4.7–22.9 cm) were used for behavioral study, representing neonates to adults (Bigelow and Schroeder, 1953; de Carvalho, 1999). Animals were obtained from trawls at Cape Canaveral and Panacea, FL, or captured using hand-nets at Long Key, FL. Neonates were obtained after one female gave birth in captivity. Animals were housed in two 3625 l flow-through aquaria (2.44 m × 1.22 m × 1.22 m) at the Florida Atlantic University Marine Laboratory (Boca Raton, FL, USA). They were maintained on a 12L: 12D light cycle. Rays were initially fed daily to satiation with live clamworms or bloodworms, then weaned to accept a diet of thawed squid and shrimp. Daily feeding behavior was monitored both visually and electrically. Electric activity in the tank was recorded using two pairs of stainless-steel electrodes (5 cm × 1 cm) positioned transversely (2.44 m apart) and longitudinally (1.22 m apart) along the walls of the tank. The electrode pairs fed directly to the differential inputs of a Warner DP-304 amplifier (Warner Instruments, Hamden, CT, USA). Signals were amplified (100 ×), filtered (0.1 Hz–10 kHz), and digitized with a PowerLab/4SP data acquisition system (ADInstruments, Sydney, Australia) sampling at 40 kHz.

Use in defense

Rays that had been regularly feeding were subjected to a simulated predatory event. The EODs were recorded with stainless-steel electrodes (2.5 cm × 2.5 cm) embedded in the fingers and thumb of an insulated rubber glove. The lead from each electrode was first fed into a voltage divider, which consisted of a 100 kΩ resistor and a 1 kΩ resistor in series to reduce the output voltage to one hundredth of the input value. This was necessary as the analog-to-digital converter has a maximum dynamic range of 10 V, and based on EODs obtained by Mathewson et al. (1958), we expected adult discharges of at least 25 V. All signals were filtered (0.1 Hz–10 kHz) and digitized with a PowerLab/4SP data acquisition system sampling at 40 kHz. The leads from the voltage divider were used as differential inputs into a Warner DP-304 amplifier. Additionally, signals from neonates were amplified (100 ×). Behavioral responses were also video recorded using a Sony Mini DV camera (Sony Corp., Tokyo, Japan). Output from the voltage divider fed into the audio input of the camera (maximum dynamic range 16 bit, 48 kHz, 3 V) as well as the PowerLab/4SP digitizer. As a result, when an EOD was emitted, an audible "pop" in the audio track enabled us to correlate the EOD with the behavior exhibited by the ray on the video track (Lowe et al., 1994).

A single ray was transferred from the main holding tank into an isolation tank (124.0 cm × 61.0 cm × 30.5 cm). The experimenter, wearing the electrode-embedded gloves, reached into the tank and grasped the ray with the electrodes contacting the dorsal and ventral surface of the MEO until the discharging ceased. Each ray was tested once. Responses were viewed in real time on the computer, and consisted of trains of several EOD spikes. These trains were also emitted in multiples, with this entire sequence being termed a set (Fig. 2).

We measured the amplitude (V) of the smallest and largest EOD discharged from each ray and quantified the number of EODs discharged per train. Amplitude was regressed against DW to test for changes in voltage with size or age. After applying a log transformation, a Student's *t*-test was performed to test for differences in the number of EODs per train between neonates (DW < 7.5 cm) and juveniles and adults, combined

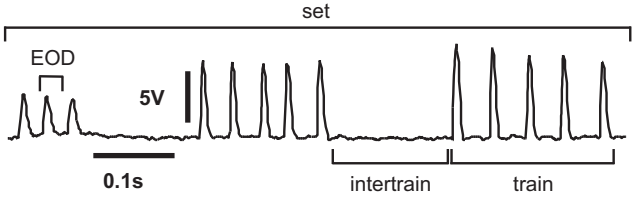

Fig. 2. A typical defensive response generated by the main electric organ of *N. brasiliensis* to explain electric organ discharge (EOD) terminology. Multiple EODs, or spikes, comprise a train. Multiple trains comprise a set. Time between trains within a set was termed the intertrain.

(DW >7.5 cm). Neonates were placed in their own group, as Michaelson et al. (1979) found unique discharge characteristics in neonatal torpedinids.

The first set of discharges for each individual was subjected to additional analysis. Within this first set, we tested for differences in train and intertrain duration between the first and last trains. Similarly, we applied a fast Fourier transform and tested for differences in fundamental frequency between the two trains. Repeated-measure ANOVAs were performed to test for differences between the first and last of each of these variables for all individuals. We also compared the discharges of neonates, and juveniles and adults separately by employing two-tailed, independent-sample *t*-tests. Train and intertrain duration data were log transformed to achieve normality.

Use in predation

Each ray was placed in an isolation tank (124.0 cm × 61.0 cm × 30.5 cm) with a 4 cm deep sand layer as substrate. Three live polychaetes (*Nereis virens*), the most common prey item of *N. brasiliensis* (Goitein et al., 1998), were buried in the sand to create a semi-natural setting for *N. brasiliensis*. Rays were not fed for 3 days to maximize motivation to feed. The rays were filmed and electrical activity in the tank was recorded before and during the experiment.

Two pairs of stainless-steel electrodes (5 cm × 1 cm) were positioned transversely and longitudinally along the walls of the tank to differentially record EODs. One pair of electrodes fed directly to the amplifier, digitizer, and computer to be recorded using the same parameters as listed above for the defensive experiments. The second pair of electrodes fed into the voltage divider, then to the audio input of the video camera as well as the PowerLab/4SP digitizer. Each ray successfully demonstrated at least three predation events. Prey ingestion was confirmed by sifting through the sand at the end of the experiment to locate any remaining worms.

Results

Morphometrics

No statistically significant differences were found between the fresh and preserved specimens, thus enabling us to pool data for the following characteristics: surface area of the MEO ($p = 0.30$, df = 2, $F = 1.117$), masses of the MEO ($p = 0.061$, df = 2, $F = 3.834$) and AEO ($p = 0.374$, df = 2, $F = 0.816$), and ESI for the MEO ($p = 0.42$, df = 2, $F = 0.679$) and AEO ($p = 0.587$, df = 2, $F = 0.303$).

The number of columns of electrocytes in the MEO increased linearly with DW (Fig. 3, open circles; $y = 6.88x + 257.48$, $R^2 = 0.26$, $p < 0.01$, $F = 8.91$, df = 1), whereas the surface area of the MEO increased as a power function (Fig. 3, closed circles; $y = 0.19x^{1.96}$, $R^2 = 0.97$, $p < 0.01$, $F = 829.18$, df = 28).

The masses of both the MEO and AEO of *N. brasiliensis* exhibited positive power relationships with DW (Fig. 4; MEO: $y = 0.024x^{2.72}$, $R^2 = 0.96$, $F = 435.44$, df = 28; AEO: $2 \cdot 10^{-5}x^{3.52}$, $R^2 = 0.92$, $F = 311.64$, df = 12) possessed MEOs and AEOs of significantly greater mass than the females ($n = 18$; ANCOVA, with DW as the covariate.

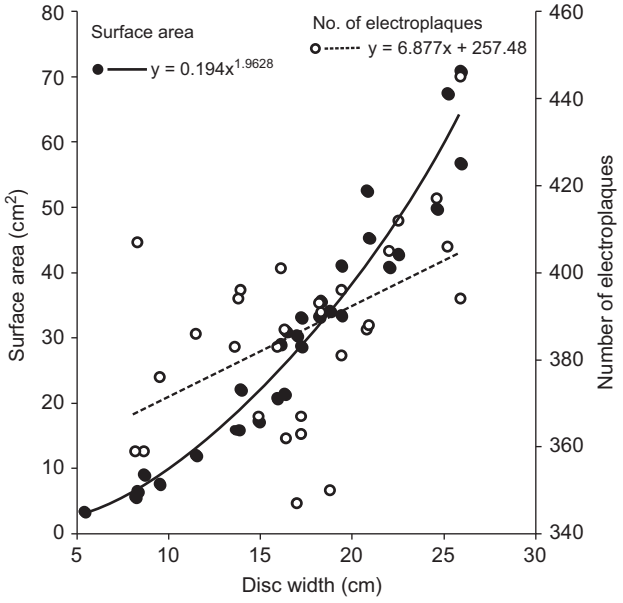

Fig. 3. For the main electric organ, both the surface area (filled circles, solid line) and the number of columns of electrocytes (open circles, dashed line) scale positively with disc width.

MEO: $p<0.01$, $F=75.95$, df = 2; AEO: $p<0.01$, $F=41.014$, df = 2; Fig. 4).

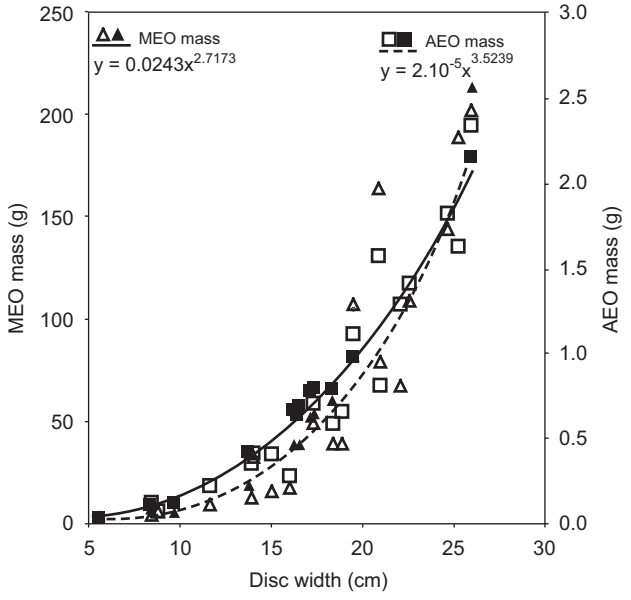

Fig. 4. Both the main (triangles, solid line) and the accessory (squares, dashed line) electric organs grow as power functions in relation to disc width. Data from males are shown in filled triangles and squares, data from females are shown in open triangles and squares.

The MEO mass relative to the body mass (MESI) did not change significantly with DW (ANOVA: $p=0.59$, $F=0.31$, df = 1, $n=30$). However, the AESI increased significantly with DW (ANOVA: $p<0.01$, $F=10.483$, df = 1, $n=30$). The AESI for males ($n=12$) was significantly greater than for females ($n=18$; ANCOVA: $p<0.01$, $F=7.16$, df = 2); however, the MESI showed no such sexual dimorphism (ANCOVA: $p=0.41$, $F=0.93$, df = 2).

Behavioral function

Use in defense

Every grasped specimen ($n=29$) emitted EODs in a pattern of several sets of multiple trains (see the supplementary movie at doi:10.1016/j.zool.2009.02.002). Discharges were accompanied by escape attempts in which the ray flexed and curled its tail both dorsoventrally and laterally. Whereas these behaviors were consistent in all rays tested, the EOD characteristics of the neonates differed from those of the adults and juveniles.

EOD characteristics

Discharges varied in amplitude between and within trains (Table 1). We quantified the maximum and

Table 1. Main EOD characteristics for two age classes of *N. brasiliensis*: neonates, and juveniles and adults combined.

	Train duration[a] (ms)			**Intertrain duration[b] (ms)**			**Train frequency[c] (Hz)**		
	First	**Last**		**First**	**Last**		**First**	**Last**	
Neonates	70 ± 10	520 ± 180	**$p<0.01$**	690 ± 140	610 ± 200	**NS, $p=0.11$**	192.9 ± 7.01	151.2 ± 12.53	**$p<0.05$**
			$F=14.9$, df = 1, $n=18$[d]			$F=3.10$, df = 1, $n=13$[d]			$F=8.53$, df = 1, $n=18$
Juveniles & adults	40 ± 10	110 ± 30	**$p<0.01$** $F=10.08$, df = 1, $n=11$[d]	530 ± 110	130 ± 30	**$p<0.01$** $F=78.40$, df = 1, $n=9$[d]	136.5 ± 17.79	110.6 ± 15.61	**$p<0.05$** $F=6.79$, df = 1, $n=11$
	$p<0.05$	**$p<0.05$**		**NS, $p=0.92$**	**$p<0.05$**		**$p<0.01$**	**$p<0.05$**	
	$F=7.131$, df = 1, $n=28$[d]	$F=7.187$, df = 1, $n=22$[d]		$F=0.011$, df = 1, $n=28$[d]	$F=4.410$, df = 1, $n=22$[d]		$F=10.306$, df = 1, $n=28$[d]	$F=7.131$, df = 1, $n=22$[d]	

Mean values and standard error of the mean are provided for each variable. An ANOVA was applied to test for differences between the first and last variable of each treatment (p-values are provided in the cells to the right of each first and last comparison within an age group). An ANOVA was also applied to test for differences between the two age classes for each variable (p-values are provided in the cells directly below each neonate, and juvenile and adult comparison for each variable).

df = degrees of freedom; NS = not significant.

[a]Time for one train of several EODs.

[b]Time between two trains of EODs.

[c]Repetition rate of EODs within a train.

[d]Log transformation applied.

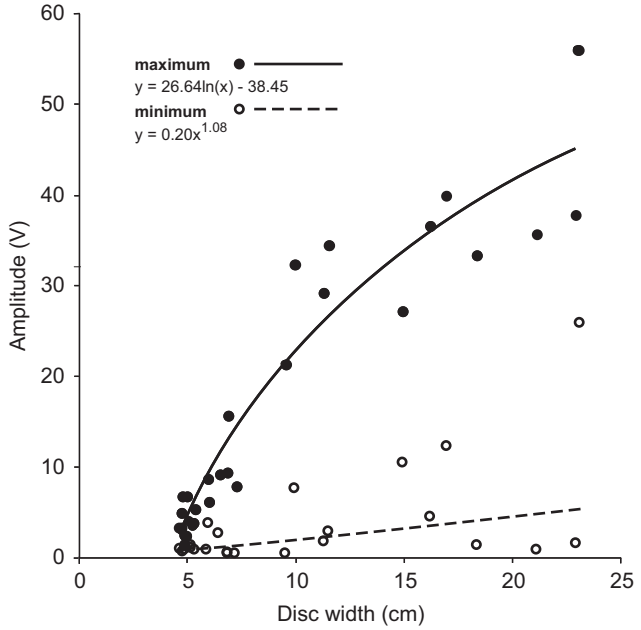

Fig. 5. Amplitude of the smallest (dashed line, open circles) and largest (solid line, filled circles) EODs recorded from *N. brasiliensis*. EOD measurements from neonates (disc width <7.5 cm) were obtained 2 days after birth.

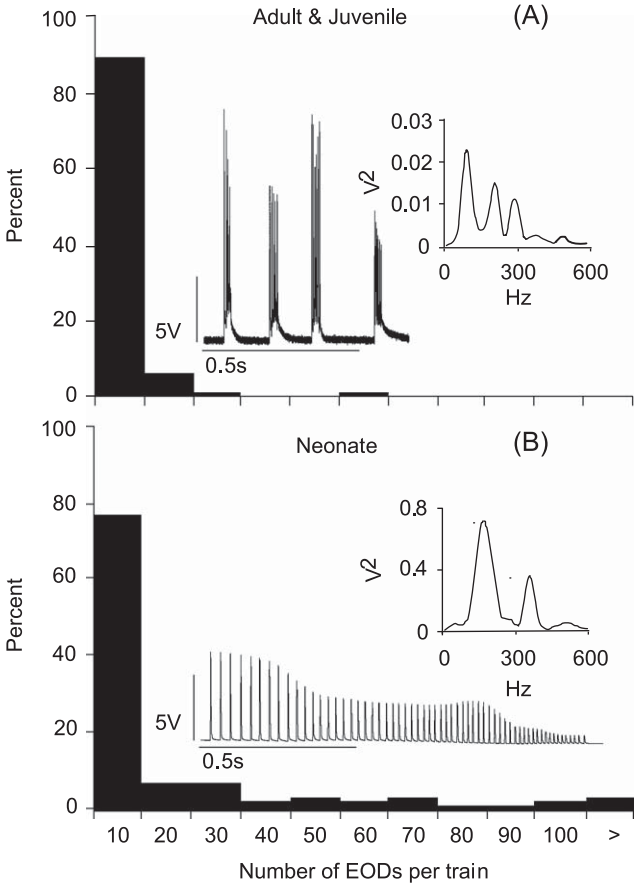

Fig. 6. Histograms illustrating the distribution of EODs per train. Most EOD trains were comprised of fewer than 10 discharges. Adults and juveniles (A) discharged a significantly lower number of EODs per train than neonates (B). A typical short adult EOD train and a long neonate train, and their fast Fourier transforms are shown in the insets.

minimum EOD amplitudes observed, both of which were positively correlated with DW (Fig. 5). Maximum EOD amplitude demonstrated a positive logarithmic relationship with DW ($y = 26.64\ln(x)–38.45$; $R^2 = 0.91$, $p<0.01$, $F = 177.55$, df = 1; Fig. 5, closed circles, solid line). Although the positive power relationship between minimum EOD amplitude and DW was significant ($y = 0.20x^{1.07}$; $R^2 = 0.38$, $p<0.01$, $F = 16.79$, df = 1; Fig. 5, open circles, dashed line), there was much more variability than in the maximum amplitude.

Neonates discharged a significantly greater number of EODs per train (15.0 ± 2.32 SE, $n = 160$) than did the juveniles and adults (6.1 ± 0.44 SE, $n = 349$; Student's *t*-test, log-transformed data, two tailed: $p<0.01$, df = 507; Fig. 6). Neonates also demonstrated a greater variation in the number of EODs per train compared with adults (Fig. 6).

The duration of the first train of the first set was significantly shorter than the duration of the last train of the first set for all individuals combined, neonates alone, and adults and juveniles combined (Table 1). Neonates discharged both first ($p<0.05$, df = 1) and last ($p = 0.05$, df = 1) trains that were significantly longer than those of adults and juveniles (Table 1).

For all individuals combined, the first intertrain duration was significantly longer than the last ($p<0.05$, df = 1). However, when analyzed by age class, these differences were not seen in the neonates, but there were significant differences with the juveniles and adults (Table 1). There was no difference in the duration of first intertrains between the two age classes ($p = 0.92$, df = 1); however, the last intertrain was significantly longer in the neonates than the adults and juveniles combined ($p<0.05$, df = 1; Table 1).

Fundamental frequency of the first train was significantly higher across all individuals, both combined ($p<0.05$, df = 1) and when separated into age classes (Table 1). Between the two age classes, neonates had significantly higher first ($p<0.01$, df = 1) and last train frequencies than adults and juveniles combined ($p<0.05$, df = 1; Table 1). This can be seen in the Fourier transforms of two exemplary trains of EOD inset in Fig. 6.

Use in predation

All worms were consumed in each trial (three per ray). However, none of the rays ($n = 29$) produced electrical discharges while searching for or consuming prey. Moreover, every daily feeding was observed over several months, and discharges were never observed for either live or thawed prey items.

Discussion

This study is the first to integrate electric organ morphology and physiology with behavioral observations of the lesser electric ray. Our findings on the ontogenetic changes of electric organs also contribute to an understanding of the behavioral function of each type of electric organ.

The MEO is composed of hundreds of columns acting in parallel, each containing hundreds of electrocytes acting in series (Bass, 1986; Zupanc and Bullock, 2005). Past studies (Cox and Breder, 1943; Mathewson et al., 1958) determined that the average voltage generated by an electrocyte is 0.35 mV and that the average amperage generated by the organ is approximately 0.45 A/cm^2. We have demonstrated that approximately seven columns of electrocytes are added to the MEO for each centimeter of DW growth. In contrast to this linear relationship, the entire organ's surface area increases as a power function (Fig. 3), therefore indicating that the columns of electric cells not only increase in number but also in size throughout ontogeny. Consequently, we can infer that the greater current measured in larger electric organs (Cox and Breder, 1943) is mostly attributable to an increased diameter of electrocytes, rather than the increased number of columns of electrocytes.

The two types of electric organ grow at different rates in *N. brasiliensis*. Whereas the AESI is positively allometric, the MESI does not change with DW. Additionally, there is sexual dimorphism in the ESIs, such that the males possess greater proportions of both types of organs than the females. Sexual dimorphism in electric organ masses has also been found in the skate, *Leucoraja erinacea* (Morson and Morissey, 2007). This skate has been shown to use these organs in intraspecific communication (Bratton and Ayers, 1987). Similarly, we propose that our morphometric data support the hypothesis that the AEO is used in communication. This could aid these rays in identification of conspecifics, since they spend much of their time buried in the substrate or in murky water (Rudloe, 1989; Rudloe and Rudloe, 1993). Moreover, the smaller MESI in these aplacental viviparous females (Bigelow and Schroeder, 1953; Devadoss, 1998) may be a potential trade-off in resource allocation. Future experiments on the social behavior of *N. brasiliensis* should and can be performed in similar laboratory conditions.

MEO use in defense and EOD characteristics

All individuals in this study produced main EODs as a defensive response. From the defensive recordings, we were able to perform a detailed analysis of the EOD, discharge patterns, and amplitude throughout ontogeny. In this study, the maximum amplitude recorded is 56 V, from a ray with a DW of 22.9 cm. The previously recorded maximum amplitude of 35 V for *N. brasiliensis* was obtained from a ray of 14 cm DW (Cox and Breder, 1943). By inserting the DW of this ray into our model (Fig. 5), we obtain a very similar value of 32 V. Moreover, our study is the first to incorporate the minimum EOD amplitudes produced by *N. brasiliensis*. Although more variable than the maxima, the minima exhibit a significant positive relationship with DW (Fig. 5).

Previous studies of defensive behavior of torpedinids, in which the ray was struck with a rod to elicit a response (Belbenoit, 1986), report trains of fewer than 20 EODs. In this study, we demonstrate that juveniles and adults commonly discharge few EODs in each train, yet are capable of discharging longer trains of more than 50 (Fig. 6a). Neonates also commonly discharge short trains; however, they also produce trains consisting of over 200 discharges. We posit that adults may discharge fewer EODs when defending themselves, as a few strong discharges may suffice to deter predators. Conversely, neonates may be more willing to discharge longer trains until fatigued, as their weaker EODs may be a less effective predator deterrent. Michaelson et al. (1979) report similar findings in the closely related *Torpedo ocellata*, where "irritated" neonates discharged relatively more EODs per train (up to eight) than the adults (fewer than six).

In contrast to previous studies describing EODs from torpedinids and narcinids (Cox and Breder, 1943; Mathewson et al., 1958; Bennett and Grundfest, 1961; Michaelson et al., 1979; Belbenoit, 1979, 1986), the patterns of EODs obtained in our study are likely a more accurate depiction of a natural, defensive response in the wild, as the grasp was performed specifically to mimic an attack by a predator.

This study is also the first to analyze patterns of train duration, intertrain duration, and fundamental frequency during a defensive response. The adults discharge at approximately 75% of the rate of neonates (Table 1). The frequencies observed from *N. brasiliensis*, for both adults and neonates, range from 19 to 305 Hz, with most being generated at less than 200 Hz. This differs from previous studies of the closely related *Torpedo marmorata*, which is reported to discharge at average rates of approximately 350 Hz (Belbenoit, 1979). Our data do provide support for the hypothesis proposed by Belbenoit (1979), who describes the neuronal circuitry controlling neonatal EODs. EODs are produced as a result of excitatory postsynaptic potentials (EPSPs) in the command nucleus, which, in turn, causes EPSPs at the electrocyte level, thus resulting in the discharge of EODs (Albe-Fessard and Buser, 1955; Szabo, 1957, 1961). After the initial short EPSP at the command nucleus, a relay nucleus initiates a chemically mediated feedback loop acting on the command nucleus, which then generates more EPSPs.

These EPSPs are approximately double the initial EPSP's duration. This increase in duration allows for a greater number of neurons in the command nucleus to be triggered, which results in a greater number of electrocytes being activated and thus discharging an EOD with increased amplitude (Szabo, 1957; Belbenoit, 1979). Belbenoit (1979) proposed that in the torpedinid neonatal system, the EPSPs are generated at a rate that is too fast to produce large-amplitude EODs; rather, it produces smooth, long trains of weak pulses, as observed in the neonates in this study.

We also found that EODs are generated at a higher frequency and for a shorter period of time at the start of a set (Table 1). These patterns are similar to those found in the torpedinids *T. californica* (Lowe et al., 1994) and *T. marmorata* (Belbenoit, 1979). It is thus very likely that, like the torpedinids, narcinids also exhibit these patterns as a result of fatigue of the chemically mediated synapses producing the EPSPs (Belbenoit, 1979).

Although we cannot discount the possibility of the AEO discharging concurrently, the signal amplitude from the AEO is so small (less than 100 mV; Bennett and Grundfest, 1961) that it would not have any significant effects on the results for MEODs.

MEO use in predation

We predicted that the small infaunal prey items of *N. brasiliensis* would not necessitate the use of the MEO to subdue prey. Indeed, we found that no EODs were recorded during live feeding events. This contrasts with the behavioral function of the large electric organs of *T. californica* (Bray and Hixon, 1978; Lowe et al., 1994). This torpedinid feeds on larger fish and consequently, immobilization of its prey is necessary for prey processing and ingestion (Lowe et al., 1994; Neer and Cailliet, 2001). In their torpedinid studies, Lowe et al. (1994) and Belbenoit (1981) both concluded that the mechanical stimulation of prey moving beneath the ray is required to elicit an EOD. However, worms buried beneath the sand may provide insufficient mechanical stimulation to elicit the same EOD response from *N. brasiliensis*.

Conclusions

This study quantified the morphological and physiological changes in the electric organs of *N. brasiliensis* throughout ontogeny to test hypotheses about their behavioral function. Our AEO morphological data provide preliminary data for future experiments directly testing the AEO's behavioral function in intraspecific communication. This study demonstrates that throughout ontogeny *N. brasiliensis* exhibits main EOD patterns and defensive responses similar to those of the Torpedinidae. However, unlike the torpedinids, which discharge during predation, we demonstrated that *N. brasiliensis* does not. Rather, this electric ray appears to utilize its strong EODs solely in defense.

Acknowledgements

We thank the Elasmobranch Research Laboratory at Florida Atlantic University for assistance with set-up and execution of experiments, data analysis, and for comments on an earlier draft of the manuscript. Both C. Lowe and M. Salmon were highly instrumental in providing comments on an earlier draft of the manuscript and in the development of the experimental design. We also thank Jack and Anne Rudloe of the Gulf Specimen Marine Lab for supplying both live and frozen specimens. G. Burgess at the Florida Museum of Natural History graciously provided preserved specimens. Several live specimens were acquired through J. Shenker and K. Johnson of the Florida Institute of Technology. Special thanks to M. Dean for assistance with husbandry. Seabait Inc. (Maine) provided the clamworms used in predatory trials. We acknowledge the continued support of Dusky Marine. This work was funded by the Donald R. Nelson Behavioral Research Award (American Elasmobranch Society), the West Palm Beach Fishing Club, and FAU Internal Funding.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.zool.2009.02.002

References

Albe-Fessard, D., Buser, P., 1955. Analyse microphysiologique des mécanismes de commande de la décharge chez la Torpille. In: Microphysiologie comparée des éléments excitables, Vol. 67. Centre National de la Recherche Scientifique, Paris, pp. 305–324.

Alves-Gomes, J.A., 2001. The evolution of electroreception and bioelectrogenesis in teleost fish: a phylogenetic perspective. J. Fish Biol. 58, 1489–1511.

Bass, A.H., 1986. Electric organs revisited. In: Bullock, T.H., Heiligenberg, W. (Eds.), Electroreception. Wiley, New York, pp. 13–70.

Belbenoit, P., 1979. Electric organ discharge of *Torpedo* (Pisces); basic pattern and ontogenetic changes. J. Physiol. Paris 75, 435–441.

Belbenoit, P., 1981. Sensory control of predatory and defensive behaviours in *Torpedo marmorata* (Pisces). In: Szabo, T., Czeh, G. (Eds.), Advances of Physiological Sciences, vol.

31: Sensory Physiology of Aquatic Lower Vertebrates. Pergamon Press and Akademiai Kiado, Budapest, pp. 235–254.

Belbenoit, P., 1986. Fine analysis of predatory and defensive motor events in *Torpedo marmorata* (Pisces). J. Exp. Biol. 121, 197–226.

Belbenoit, P., Bauer, R., 1972. Video recordings of prey capture behaviour and associated electric organ discharge of *Torpedo marmorata* (Chondrichthyes). Mar. Biol. 17, 93–99.

Bennett, M.V.L., 1971. Electric organs. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, vol. 5. Academic Press, New York, pp. 347–349.

Bennett, M.V.L., Grundfest, H., 1961. The electrophysiology of electric organs of marine electric fishes. II. The electroplaques of main and accessory organs of *Narcine brasiliensis*. J. Gen. Phys 44, 805–818.

Bennett, M.V.L., Wurzel, M., Grundfest, H., 1961. The electrophysiology of electric organs of marine electric fishes. I. Properties of electroplaques of *Torpedo nobiliana*. J. Gen. Phys 44, 757–804.

Bigelow, H.B., Schroeder, W.C., 1953. In: Fishes of the Western North Atlantic. Part 2. Sawfishes, Guitarfishes, Skates and Rays; Chimaeroids. Sears Foundation for Marine Research, New Haven.

Bratton, B.O., Ayers, J.L., 1987. Observations on the electric organ discharge of two skate species (Chondrichthyes, Rajidae) and its relationship to behavior. Exp. Biol. Fish 20, 241–254.

Bray, R.N., Hixon, M.A., 1978. Night-shocker: predatory behavior of the Pacific electric ray (*Torpedo californica*). Science 200, 333–334.

Coates, C.W., 1934. Some electric fishes at the aquarium. Bull. NY Zool. Soc. 37, 199–208.

Cox, R.T., Breder, C.M., 1943. Observations on the electric discharge of *Narcine brasiliensis* (Olfers). Zool. Scr. 26, 45–51.

de Carvalho, M.R., 1999. A systematic revision of the electric ray genus *Narcine* Henle, 1834 (Chondrichthyes: Torpediniformes: Narcinidae) and the higher-level phylogenetic relationships of the orders of elasmobranch fishes (Chondrichthyes). Ph.D. Thesis, Department of Biology, The City University of New York, New York.

Dean, M.N., Motta, P.J., 2004. Feeding behavior and kinematics of the lesser electric ray, *Narcine brasiliensis* (Elasmobranchii: Batoidea). Zoology 107, 171–189.

Devadoss, P., 1998. Observations on the breeding and development in some batoid fishes. Indian J. Fish 45, 271–283.

Goitein, R., Torres, F.S., Signorini, C.E., 1998. Morphological aspects related to feeding of two marine skates *Narcine brasiliensis* Olfers and *Rhinobatus horkelli* Muller and Henle. Acta Sci. 20, 165–169.

Lowe, C.G., Bray, R.N., Nelson, D.R., 1994. Feeding and associated electrical behavior of the Pacific electric ray *Torpedo californica* in the field. Mar. Biol. 120, 161–169.

Mathewson, R., Mauro, A., Amatniek, E., Grundfest, H., 1958. Morphology of main and accessory electric organs of *Narcine brasiliensis* (Olfers) and some correlations with their electrophysiological properties. Biol. Bull. 115, 126–135.

Michaelson, D.M., Sternberg, D., Fishelson, L., 1979. Observations on the feeding, growth, and electric discharge of newborn *Torpedo ocellata* (Chondrichthyes, Batoidei). J. Fish Biol. 15, 159–163.

Moller, P., 1995. In: Electric Fishes: History and Behavior. Chapman & Hall, New York.

Morson, J.M., Morissey, J.F., 2007. Morphological variation in the electric organ of the little skate (*Leucoraja erinacea*) and its possible role in communication during courtship. Environ. Biol. Fish. 80, 267–275.

Neer, J.A., Cailliet, G.M., 2001. Aspects of the life history of the pacific electric ray, *Torpedo californica* (Ayres). Copeia 2001, 842–847.

Nelson, J.S., 1994. In: Fishes of the World, third ed. Wiley, New York.

Pfeiffer, W., 1961. Zur Biologie des Zitterrochens (*Torpedo marmorata* Risso). Publ. Staz. Zool. Napoli 32, 67–71.

Rasband, W.S., 1997. ImageJ. US National Institutes of Health, Bethesda, MD, USA.

Rudloe, A., 1989. Habitat preference, movement, size frequency patterns and reproductive seasonality of the lesser electric ray, *Narcine brasiliensis*. Northeast Gulf Sci. 10, 103–112.

Rudloe, A., Rudloe, J., 1993. Electric warfare: the fish that kill with thunderbolts. Smithsonian 24, 94–105.

Stoddard, P.K., 1999. Predation enhances complexity in the evolution of electric fish signals. Nature 400, 254–256.

Szabo, T., 1957. Anatomo-physiologie comparée de divers organs électriques et de leurs centres nerveus de commande. Thesis, University of Paris, Paris.

Szabo, T., 1961. Anatomo-physiologie des centres nerveux spécifiques de quelques organs électriques. In: Chagas, C., Carvalho, P. (Eds.), Bioelectrogenesis. Elsevier, Amsterdam, pp. 185–201.

Wilson, D., 1953. Notes from the Plymouth Aquarium II. J. Mar. Biol. Assoc. UK 32, 199–208.

Zupanc, G.K.H., Bullock, T.H., 2005. Electrogenesis to electroreception: an overview. In: Bullock, T.H., Hopkins, C.D., Popper, A.N., Fay, R.R. (Eds.), Electroreception. Wiley, New York, pp. 5–46.