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ARTICLE

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Abstract

The objective of the present study was to describe and characterize macroscopic and microscopic aspects of the reproductive biology of the Giant Electric Ray *Narcine entemedor*, a viviparous elasmobranch targeted by commercial fishers in Mexico. A total of 305 individual rays were captured (260 females, 45 males); all males were sexually mature. The median size at maturity for females was estimated to be 58.5 cm TL, the median size at pregnancy was 63.7 cm TL, and the median size at maternity was 66.2 cm TL. The range of ovarian follicles recorded per female was 1–69; the maximum ovarian fecundity of fully grown vitellogenic oocytes was 17, and uterine fecundity ranged from 1 to 24 embryos per female. The lengths of the oblong ovarian follicles varied significantly among months, and the largest ovarian follicles were found in July, August, and September. Median embryo size was largest in August, and the size at birth was between 12.4 and 14.5 cm TL. Histological evidence of secretions from the glandular tissue of the uterine villi indicate that this species probably has limited histotrophy as a reproductive mode. Vitellogenesis in the ovary occurred synchronously with gestation in the uterus. The Giant Electric Ray has a continuous annual reproductive cycle; a period of ovulation occurs between May and September and two peaks of parturition, one in January and one in August, occur, suggesting that embryonic diapause occurs in some individuals. These results provide useful information for the management of this important commercial species in Bahía de La Paz, Mexico, and will allow possible modification of the current Mexican regulations to enable better protection of this species.

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The elasmobranchs (sharks, skates, and rays) present diverse modes of reproduction that have contributed to the success of this group for more than 400 million years (Helfman et al. 1997:151–178). Wourms (1981) proposed two reproductive modes, based on the type of embryonic nutrition: lecithotrophy, where the embryos depend exclusively on yolk, and matrotrophy, where in addition to yolk the mother secretes other nutritious substances. Additionally, the group has developed diverse reproductive tactics, and some species exhibit embryonic diapause (i.e., an interruption of embryonic development during gestation as defined by Simpfendorfer 1992), sperm storage in females (Pratt 1993; Pratt and Carrier 2001; Waltrick et al. 2014), and selection of specific sites for parturition. Such sites are known as nursery areas and provide protection against predators to ensure better chances of survival of their offspring (Hueter et al. 2004).

The electric rays (Order Torpediniformes) have been defined as yolk sac viviparous (lecithotrophs; Ranzi 1932, 1934; Hamlett et al. 2005). However, Villavicencio-Garayzar (2000) reported that the Giant Electric Ray *Narcine entemedor*, in the lagoon complex of Bahía Almejas, Baja California Sur, Mexico, has a viviparous matrotrophic reproductive mode and exhibits embryonic diapause. Several species in this order have two functional ovaries and uteri, e.g., Ocellated Torpedo *Torpedo torpedo* (Capapé et al. 2000), Pacific Electric Ray *T. californica* (Neer and Cailliet 2001), and Lesser Electric Ray *N. brancoftii* (Moreno et al. 2010), and all species in the order lack the oviducal gland (Prasad 1945).

The Giant Electric Ray is distributed from the Bahía Magdalena, on the west coast of Baja California Sur, including the Gulf of California, southward to Peru (Robertson and Allen 2015). This is one of the species most frequently captured by artisanal fishers in northwestern Mexican waters and is opportunistically fished throughout the year (Villavicencio-Garayzar 2000; Márquez-Farías 2002). However, the agency responsible for management of sharks and batoids in Mexico (INAPESCA, acronym in Spanish) established a closed season between May 1 and July 31 prohibiting the capture of elasmobranchs in the Mexican Pacific Ocean and sharks in the Gulf of Mexico (Official Mexican Standard for Fishing, NOM-029-PESC-2006; DOF 2007, 2012).

Considering that biological information of populations of commercial importance is essential to ensure effective management of these species (Cortés 2004; Walker 2005; Lowerre-Barbieri et al. 2011; Dulvy et al. 2014; Simpfendorfer and Wetherbee 2015), the objective of this study was to evaluate reproductive aspects of the Giant Electric Ray in Bahía de La Paz, Baja California Sur, Mexico. Specifically, we present information on the reproductive mode, tactics, and cycle as well as estimates of characteristics related to maturity, gestation, and ovulation.

METHODS

Study site and collection of specimens.—Monthly collections of Giant Electric Rays were made from October 2013 through December 2015 in the southern zone of Bahía de La Paz, located in the southern portion of the Gulf of California (24°25′17.55″N, 110°18′31.64″W), in three different fishing grounds: El Morrito, El Quelele, and Campo Rodríguez (Figure 1). Bahía de La Paz is isolated from the majority of the hydrological processes in the Gulf of California (Salinas-Gonzáles et al. 2003). Mean annual water temperatures vary from 15°C to 22°C, and mean salinity is 35‰ but can increase during summer due to intense evaporation and little freshwater inflow (Villaseñor 1979; Salinas-González et al. 2003).

The rays were captured by artisanal fishers using monofilament gill nets (100 m long, 1.5 m high, 8–10 in stretch mesh) traditionally called *chinchorros*, which are set in the afternoon at depths between 10 and 40 m over sandy bottoms and recovered the next morning. Each fish was measured for TL (cm), weighed (total weight [TW]) and eviscerated weight [EW], 0.01 kg) and the sex determined. For males, the inner clasper length (CL, cm), the grade of calcification of the clasper (calcified, partially calcified, not calcified), and the presence or absence of semen was recorded. Gonads were macroscopically staged to define maturity, weighed (gonad weight [GW], 0.001 g), and fixed in 10% buffered formalin.

Sex ratio, length, and weight.—The sex ratio of adults and juveniles (combined) and embryos was evaluated with a chi-square test to determine whether it differed from 1:1 (Sokal and Rohlf 1998). Differences in the length and weight between males and females (excluding the weight of pregnant females) were evaluated using a Mann–Whitney *U*-test. Data were tested for normality and homogeneity of variances prior to analysis with Kolmogorov–Smirnov and Lilliefors tests, respectively. All differences were considered significant if $P < 0.05$.

Macroscopic observations of reproductive structures and maturity.—Maturity of males was defined following the criteria proposed by Neer and Cailliet (2001) and Moreno et al. (2010) and adapted for *N. entemedor* based principally on the development of the testes, the presence–absence of testicular lobes, and the presence–absence of semen (Table 1). Each testis was measured (length and width, 0.001 cm); differences between the length were evaluated using a Student's *t*-test, and width of the left and right testis were evaluated using a Wilcoxon test. Additionally, the relationship between the inner length of the clasper as a function of TL was plotted.

Maturity of females was evaluated following the criteria of Martin and Cailliet (1988), Abdel-Aziz (1994), Villavicencio-Garayzar (2000), Moreno et al. (2010), Mejía-Falla et al. (2012), and Rolim et al. (2015) adapted to specific characteristics of *N. entemedor*. We defined four phases considering macroscopic characteristics of both ovary and uterus as well as

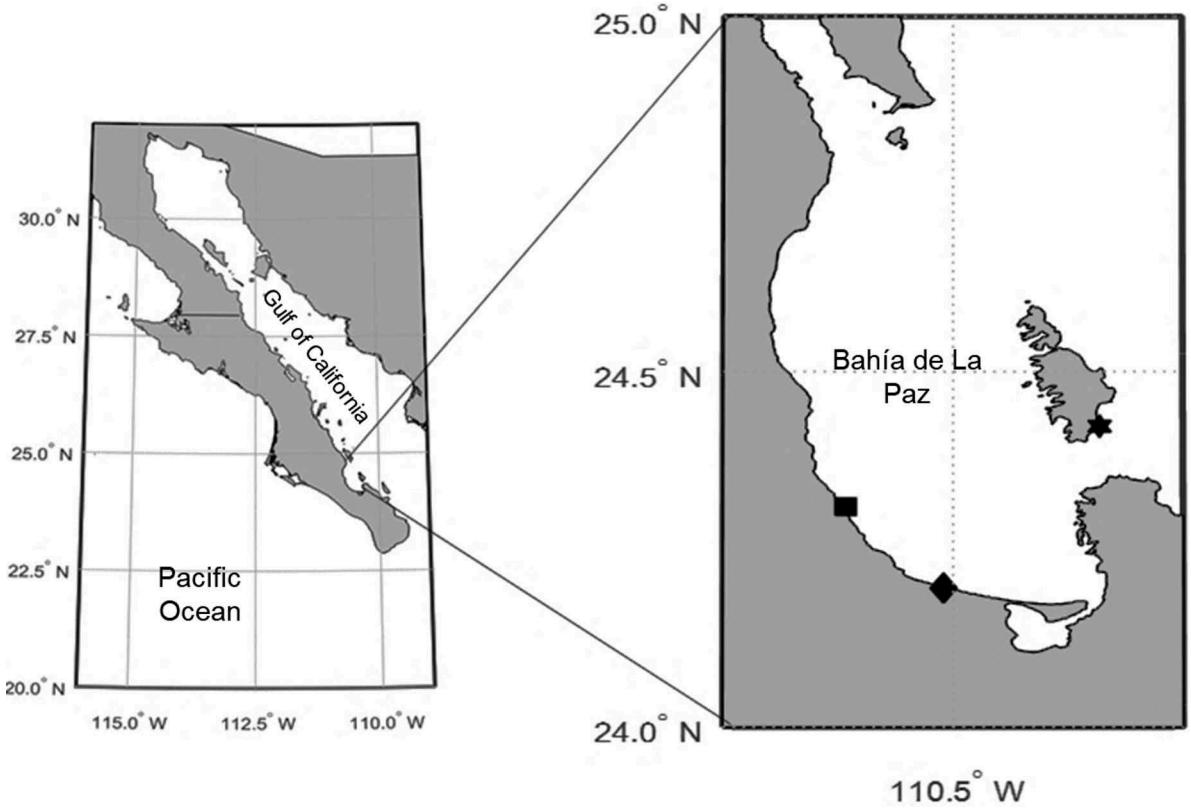

FIGURE 1. Study area (Gulf of California and Bahía de La Paz, Mexico) including primary sampling locations for Giant Electric Ray (star = El Morrito, diamond = El Quelele, square= Campo Rodriguez).

the maturity indices for each independently evaluated structure (Table 2). The width, length (0.001 cm), and weight (0.001 g) of each ovary was recorded, and the ovarian follicles were removed. The anterior oviducts and the uterus were removed and measured (width, 0.001 cm) and the presence of ovarian follicles in the anterior oviduct (completely vitellogenic as evidence of ovulation) and embryos in the uterus were recorded. The length of each villi in the uterus was measured (0.001 cm), and the abundance of villi was evaluated as few (≤50 villi) or abundant (≥51 villi). The differences in the length of the right ovary and the width of the right uterus by maturity phase were evaluated using a Kruskal–Wallis (KW) test for independent samples. Differences in the length and width between the right and left reproductive structures (ovary and uterus) of females were assessed using a Wilcoxon paired test.

Histological analysis.—Histological processing followed Burgos-Vázquez (2013) and consisted of successive changes of ethanol at increasing concentrations from 70% to 100%, followed by clearing and infiltration with paraffin in a tissue processor. Tissues were embedded in paraffin, and transverse and longitudinal sections (3–5 μm) of the ovary, anterior oviduct, and uterus of females and testis and seminal vesicles of males were stained using hematoxylin and eosin. To define male maturity, the process of spermatogenesis in the testes and seminal vesicles was examined following that defined by Maruska et al. (1996), ICES (2010), and Brown-Peterson et al. (2011). Males were considered mature when primary spermatocytes were present in the testis (Brown-Peterson et al. 2011). For females, the description of oogenesis followed that defined by ICES (2010) and Brown-Peterson et al. (2011). Slides were examined using a Nikon Eclipse 50i compound microscope and photographed with a DXM 1200C camera using ACT-1C software.

Median size of maturity, pregnancy, and maternity.—The median size at maturity (TL_{50}) for females was calculated using a logistic regression model with binomial data (0, immature; 1, mature; Table 2) with the equation

$$p_i = \left[1 + e^{-(a+b\cdot \mathrm{TL})}\right]^{-1},$$

where p_i is the fraction of mature individuals at TL, a and b are model parameters, and a/b corresponds to the median size of maturity (Mollet et al. 2000). Females were considered mature if ovaries were classified in the mature–not pregnant (3), mature–pregnant (4), or regressing (5) phases (Table 2) or if the uterus showed signs of development

TABLE 1. Macroscopic and microscopic descriptions of the male reproductive structures defined to distinguish maturity phases of the Giant Electric Ray.

			Testes			Seminal vesicle		
Maturity index	Maturity	Reproductive phase	Testes Index	Macroscopic condition	Microscopic condition	Seminal vesicle Index	Macroscopic condition	Microscopic condition
3	Mature	Mate capable	3	Presence of testicular lobules and up to 10% of epigonal tissue.	Presence of testicular lobules with spermatogonia and primary and secondary spermatocytes, spermatids and spermatozoa.	3	Differentiated vas deferens, thickened.	Without spermatozoa or seminal fluid.
4	Mature	Actively mating	4	Presence of testicular lobules and 0 to 1% of epigonal tissue.		4	Differentiated vas deferens, thickened, and the presence of semen.	With sperm and seminal fluid.

TABLE 2. Macroscopic and microscopic descriptions of the female reproductive structures defined to distinguish reproductive phases of the Giant Electric Ray.

Overall maturity			Ovaries		Uterus		
Maturity index	Maturity	Reproductive phase	Ovary index	Macroscopic condition	Microscopic condition	Uterus index	Macroscopic condition	Microscopic condition
2	Immature	Developing	2	With translucent and/or yellowish ovarian follicles (lengths < 16 cm) and abundant ovarian stroma.	With primary growth and previtellogenic ovarian follicles and abundant epigonal tissue.	2	Little differentiation in anterior oviducts, short and abundant uterine villi (<1 cm). Width, 0.2–1.2 cm.	Uterine villi of simple cylindrical epithelium, few blood vessels and little blood segregation from the basal endometrium. Lacks glandular epithelium. no histotroph secretions.
3	Mature–not pregnant	Ovulation capable	3	With yellow ovarian follicles (vitellogenic, of different lengths between 16 and 31.8 cm), little or no ovarian stroma. Follicles in different phases of the vitellogenesis, and postovulatory follicles can be present.	With primary growth, previtellogenic, and primary, secondary, and tertiary vitellogenic ovarian follicles.	3	Without eggs or embryos, with abundant uterine villi throughout the endometrium, slightly narrow and well differentiated from anterior oviducts. Width > 1.2 cm.	Uterine villi of stratified cylindrical epithelium, few blood vessels and little blood segregation from the basal endometrium. Lacks glandular epithelium, no histotroph secretions.
4	Mature–pregnant	Actively ovulating	3			4A	With eggs, lacking egg capsule, thick and abundant uterine villi. There can be ovarian follicles descending into the anterior oviduct. Width > 1.7 cm.	

TABLE 2. Continued.

Overall maturity			Ovaries			Uterus	
Maturity index	Maturity	Reproductive phase	Ovary index	Macroscopic condition	Microscopic condition	Uterus index	Macroscopic condition	Microscopic condition
		Embryos in formation	4	With translucent ovarian follicles < 16 cm long, abundant ovarian stroma and presence of postovulatory follicles.	With primary growth and previtellogenic and vitellogenic ovarian follicles, abundant epigonal tissue, atretic oocytes, and corpus luteum can be present.	4B	Lacking egg capsule, uterine walls thick with abundant uterine villi. Width > 2 cm. Embryos ≤ 3 cm with yolk sac.	Uterine villi of cylindrical stratified epitelium, with glandular tissue in the periphery of the villi; one blood vessel central in the uterine villi. Small quantity of histotroph secretion.
		Early embryos				4C	Lacking egg capsule, uterine walls thick with abundant uterine villi. Width > 2 cm. Embryos 3.1–5.8 cm with yolk sac.	
		Mid embryos	3	With yellow ovarian follicles (vitellogenic of different lengths, between 16 and 31.8 cm), little or no ovarian stroma. Follicles in different phases of vitellogenesis and postovulatory follicles can be present.	With primary growth, previtellogenic, and primary, secondary, and tertiary vitellogenic ovarian follicles.	4D	Lacking egg capsule, elongated, few uterine villi (> 1 cm), muscular walls beginning to thin, vascularized. Width ≥ 2.4 cm. Embryos 5.9–12.4 cm with reduced yolk sac.	
		Late embryos				4E	Muscular walls thin and transparent, with few villi and highly vascularized. Width ≥ 2.4 cm. Embryos > 12 cm and no yolk sac.	

TABLE 2. Continued.

Overall maturity			Ovaries			Uterus	
Maturity index	Maturity	Reproductive phase	Ovary index	Macroscopic condition	Microscopic condition	Uterus index	Macroscopic condition	Microscopic condition
5	Mature–not pregnant	Regressing	4	With translucent ovarian follicles < 16 cm long, abundant ovarian stroma and presence of postovulatory follicles.	With primary growth, previtellogenic and vitellogenic ovarian follicles and abundant epigonal tissue, atretic oocytes, and corpus luteum can be present.	5	Without eggs or embryos, with abundant uterine villi throughout the endometrium, slightly narrow, thick and well differentiated muscular walls of anterior oviducts.	Uterine villi composed of stratified cylindrical epithelium and glandular tissue on the periphery of villi. A central blood vessel in each uterine villi. No histotroph secretions.

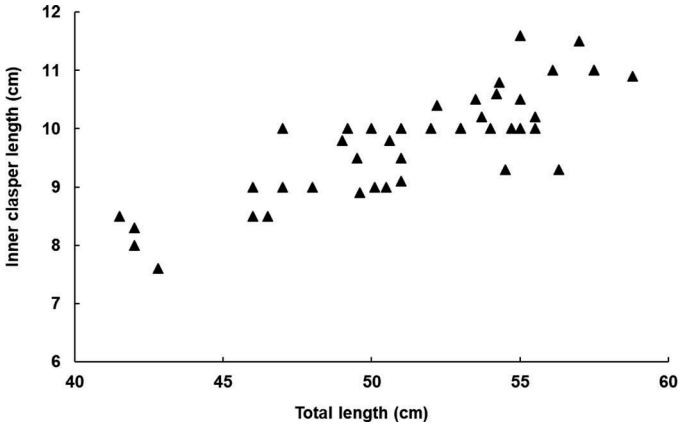

FIGURE 2. Relationship between TL (cm) and inner clasper length (cm) of male Giant Electric Rays.

(indices 3, 4A, 4B, 4C, 4D, 4E, or 5; Table 2). Similarly, the median size at pregnancy (TLP_{50}) was calculated using binomial data, where a value of 1 corresponded to females regressing or with eggs or embryos in the uterus (indices 4A, 4B, 4C, 4D, 4E; Table 2), and a value of 0 was assigned to nonpregnant females (indices 2 and 3, or 5; Table 2). For the median size at maternity (TLM_{50}), females were considered as maternal (1) if they would have produced a litter the next season, if not captured, and contained follicles ≥ 16 cm length in the months of May to December, and nonmaternal (0) if they would not have contributed offspring the next season. These values were defined from analysis of the reproductive cycle following Walker (2005) and Mejía-Falla et al. (2012).

Fecundity and reproductive cycle.—Ovarian follicles were counted and measured for length (0.001 cm). The embryos were sexed (male, female, or undetermined), measured (TL, cm), and classified ontogenetically based on morphological characteristics following Braccini et al. (2007) and modified for *N. entemedor*. We defined four stages of embryonic development: (1) In formation (embryos ≤ 3.0 cm TL with the presence of external branchial filaments, without pectoral or pelvic fins, with no coloration, and a complete and large yolk sac); (2) Early development (embryos 3.1–5.8 cm TL with defined pectoral fins, external branchial filaments present, no coloration pattern, and a large yolk sac; (3) Mid-development (embryos 5.9–12.3 cm TL without external branchial filaments, small yolk sac, defined pelvic and pectoral fins, and the beginning of coloration patterns on the skin); and (4) Late development (embryos ≥ 12.4 cm TL with developed fins, no yolk sack, and defined coloration) (Table 2).

Three different groups of ovarian follicles based on their size were defined: small (previtellogenic, sizes ranging from 0 to 5.9 cm in length), medium (early vitellogenesis, sizes between 6 and 15.9 cm in length), and large (late vitellogenesis, sizes ≥ 16 cm in length). The mean and maximum number of ovarian follicles by group were estimated, and differences among them were evaluated using a Kruskal–Wallis test. Ovarian fecundity was defined considering only large ovarian follicles. Ovarian and uterine fecundity were estimated using range, mean, and mode of the number of large ovarian follicles in the ovary and the number of embryos in the uterus, respectively (Pratt 1979).

We used linear regression to analyze the relationships between ovarian fecundity or uterine fecundity with TL. For females that presented ovarian follicles and embryos, the relationship between the length of the ovarian follicles and the size of the embryos by ontogenic stage was compared in order to evaluate the synchrony (or asynchrony) in follicular (vitellogenesis) and embryonic growth (gestation).

The reproductive cycle was evaluated in three complementary ways. First, we examined the monthly variation of the largest ovarian follicles (in length) and of the embryo size to define the months of ovulation and parturition, respectively (Walker 2005; Mejía-Falla et al. 2012). For the embryo development period, embryos ≤ 5 cm TL, belonging to pregnant females with a fecundity equal to one, were eliminated to rule out possible sampling errors (aborts or retarded growth). A Kruskall–Wallis test followed by a nonparametric Tukey post hoc test with Bonferroni adjustment (Siegel and Castellan 1988) was used to determine differences among months. Secondly, the gonadosomatic index (GSI), calculated as GSI = GW/EW × 100 (Gherbi-Barre 1983), was used. Monthly differences in GSI were evaluated using a Kruskal–Wallis test, with differences among months evaluated with a nonparametric Tukey test. Finally, the percentage of females in each reproductive phase was examined monthly across the year.

RESULTS

Sex Ratio, Length, and Weight

We analyzed a total of 305 Giant Electric Rays (260 females and 45 males), resulting in a female : male sex ratio for juveniles and adults combined of 5.7:1, which was significantly different than the expected 1:1 ratio ($\chi^2 = 151.55$, df = 1, $P < 0.001$). Females were present during all collection months and in greater abundance than males. Males were not collected in January, April, or June.

Females ranged in size from 48.5 to 84.0 cm TL (mean ± SD, 65.9 ± 7.1 cm) and males from 41.5 to 58.5 cm TL (51.4 ± 4.4 cm). Females were significantly larger ($Z = 9.95$, $P < 0.001$) and heavier ($Z = 9.96$, $P < 0.001$) than males.

Male Reproductive Structures and Reproductive Phases

Both testes in all males examined were functional, of an oval form, completely covered by the epigonal organ, and suspended in the thoracic cavity by mesenteries. There was no significant difference in the length of the left and right testis ($t = 0.142$, df = 16, $P = 0.889$), but the left testis was significantly wider (1.87 ± 0.39 cm) than the right testis (1.75

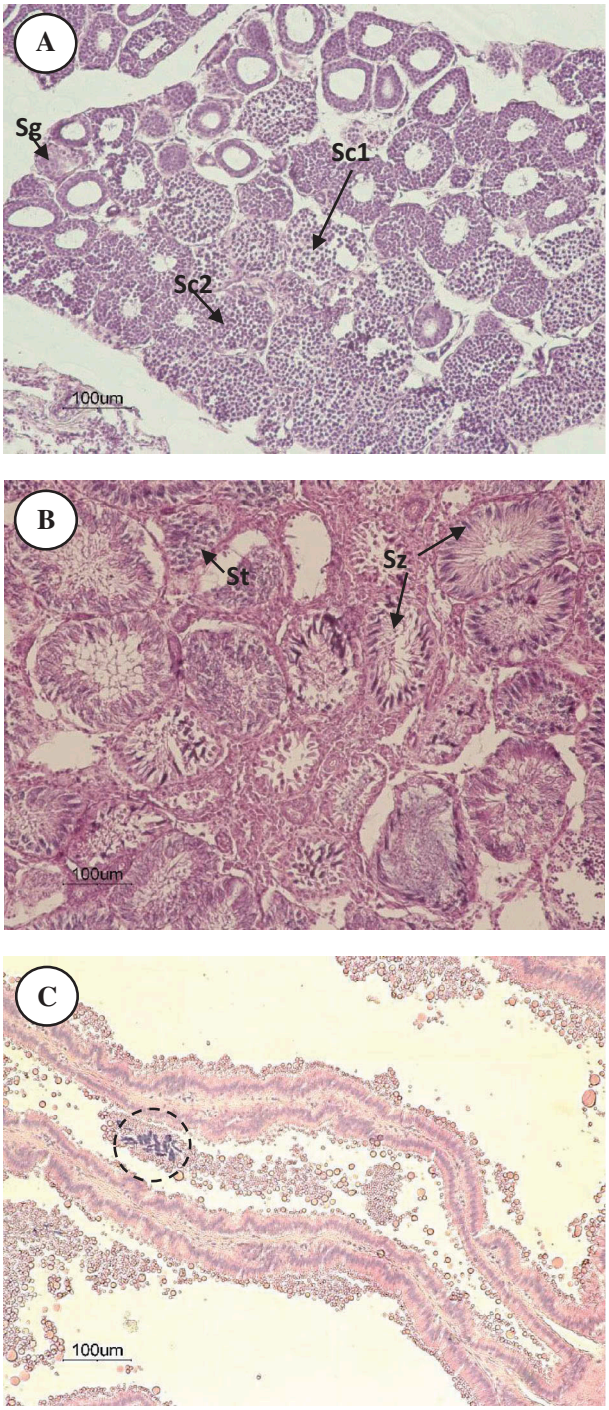

FIGURE 3. Micrographs of the reproductive structures of male Giant Electric Ray. (**A**) Longitudinal section of testes in early stages of spermatogenesis in an actively mating male. (**B**) Longitudinal section of testes in the actively mating phase. (**C**) Longitudinal section of the seminal vesicle of a male in the actively mating phase. The dotted line indicates packets of spermatozoa in a spermatozeugmata. Sg = spermatogonia, Sc1 = primary spermatocytes, Sc2 = secondary spermatocytes, St = spermatids, Sz = spermatozoa.

± 0.40 cm; $Z = 2.762$, $P = 0.005$). The claspers of all males examined were completely calcified, and the relationship between inner clasper length and male TL appeared to be linear (Figure 2).

All testes analyzed histologically ($n = 20$) had spermatocysts with different stages of spermatogenesis, with spermatogonia, primary spermatocytes, and secondary spermatocytes present in the spermatocysts (Figure 3A), and were considered sexually mature. Testes containing late stages of spermatogenesis, including spermatids and spermatozoa (Figure 3B), were identified as capable of mating (with mature spermatozoa in the testis) or actively mating if the seminal vesicles contained spermatozoa and seminal fluid. The seminal vesicles of these males also contained aggregations of spermatozoa packets of the spermatozeugmata type, with the sperm heads orientated toward the center of the packet and the tails along the margins (Figure 3C). Males in the mating-capable and actively mating reproductive phases were present during all months that males were captured. However, males in the actively mating phase dominated during July (28.9%), August (22.2%), and September (20%).

The size at sexual maturity could not be calculated for male Giant Electric Rays since no immature specimens were captured in this study. The smallest male captured (41.5 cm TL) had spermatozoa in the testis and semen in the claspers and was actively mating.

Female Reproductive Structures

All females evaluated ($n = 240$) had functional ovaries and uterus and the oviducal glands were absent. There was no significant difference between the length of the right and left ovary ($Z = 0.92$, df = 141, $P = 0.355$), but the left ovary (median = 2.85 cm) was significantly wider than the right ovary (median = 2.60 cm; $Z = 2.39$, df = 136, $P = 0.016$). There was a significant difference between the length of the right ovary and maturity reproductive phase (KW test: $H_{3,\ 152} = 68.72$, $P < 0.0001$), during which females in the ovulation-capable phase had longer ovaries (median = 5.5 cm).

Both ovaries were in the anterior portion of the thoracic cavity and suspended by the mesovarium. Ovaries are conical, and in the more advanced phases of development, follicles filled the entire ovary with little ovarian stroma remaining. All females had ovarian follicles in both ovaries. Ovarian follicles have an elongated form in which the germinal zone is located at the semispherical base and the top of the follicle is slightly pointed (Figure 4). Ovarian follicles of different lengths (0.02–31.8 cm) were present in the same ovary.

Histological analysis of the ovaries showed different gametogenic stages corresponding to the different reproductive phases. A germinal zone is evident in the periphery of the ovary near the vertebral column, and ovarian follicles are embedded in ovarian stroma, which is associated with the

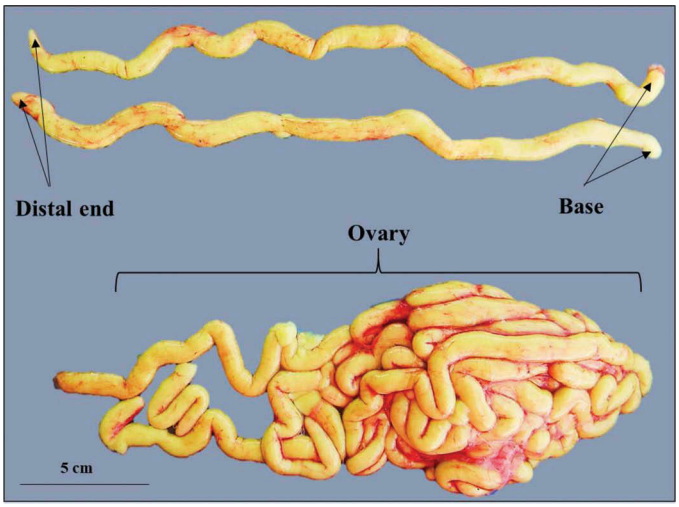

FIGURE 4. Ovarian follicles (top) and ovary (without the covering tissue) of a mature female Giant Electric Ray in the ovulation capable phase.

epigonal organ. The ovary is surrounded by a peritoneal epithelium composed of simple cylindrical tissues and collagen fibers (Figure 5A). Histological analysis permitted differentiation of the follicular epithelial layers surrounding the oocyte, i.e., the zone pelucida, theca interna, and theca externa (Figure 5B).

There were no significant differences between the width of the left and right anterior oviduct (Z = 0.95, df = 133, P = 0.338). There were significant differences in oviduct width during different reproductive phases (KW test: $H_{4,\ 161}$ = 41.112, $P < 0.0001$); ovulation-capable females had the greatest width (median = 0.45 cm) compared with other phases. The anterior oviducts have a tubular form and are connected to the anterior portion of the uterus by a slight widening of the basal portion of the oviduct. The oviduct is connected to the upper portion of the thoracic cavity by an ostium near the corner of the mouth of the esophagus. Microscopic analysis of the basal zone of the oviducts showed several disperse tubules, similar to oviductal tubules in the oviducal gland. However, the oviduct lacked the plates and secretory ducts characteristic of oviducal glands, and there was no evidence of spermatozoa in the oviduct (Figure 5C).

There were no significant differences between the left and right uteri in length (Z = 0.313, df = 162, P = 0.754) or width (Z = 1.232, df = 147, P = 0.217). However, there were significant differences among reproductive phase and uterus width (KW test: $H_{4,\ 182}$ = 91.530, $P < 0.0001$); females with a uterine index of 4A–E had a greater width (median = 4.45 cm) than females with a uterine index of 2, 3, or 5.

Uteri with an index of 2 had thin muscle walls with short and abundant uterine villi, and the anterior oviducts were not completely differentiated. Uteri with an index of 3 had a thick layer of muscle covered by a serosa layer with abundant and short uterine villi. In uteri of females in the mature–pregnant phase, the muscular tissue expanded leaving only the serosa layer, which had a venous system originating in the anterior part of the uterus, and the uterine villi were longer and more dispersed with an expansion of the endometrium. The amount of uterine villi varied with uterine index; indices 4A, 4B, and 4C presented abundant uterine villi, while few uterine villi were present in uteri with indices 4D and 4E. At the microscopic level, the uterus was composed of muscle fibers under a layer of connective tissue in all reproductive phases, but the uterine villi changed structurally according to the reproductive phase (Figure 5D, E, F, G). Uterine villi in uteri having indices of 2 and 3 were composed of simple cubical tissue with a main blood vessel (Figure 5E), while villi in those with indices 4A, 4B, 4C, and 4D were composed of stratified cylindrical tissue of approximately six layers of cells (Figure 5H). Finally, the lumen of the uterus of the pregnant females showed acidophilic secretions from the secretory crypts of each uterine villi (Figure 5H).

Female Size at Maturity, Pregnancy, and Maternity

Immature, developing females (n = 47, 18.1% of total) ranged in size from 48.5 to 69.0 cm TL, while mature females (n = 213, 81.9% of total) in the mature–not pregnant, mature–pregnant, and mature–regressing phases ranged in size from 54.5 to 85.0 cm TL. The largest immature female (69.0 cm TL) had undeveloped ovaries, ovarian follicles < 4.4 cm long, and thin uteri with very short uterine villi (uterine index 2). However, a smaller (54.5 cm TL), sexually mature female had ovarian follicles completely developed (24.5 cm in length) and narrow but completely differentiated uteri with abundant uterine villi (uterine index 3).

The TL_{50} in females based on maturity index (considering all structures) together was estimated at 58.5 cm TL (95% CI: 51.7–65.4; Figure 6A), very similar to values estimated by considering only ovarian development (58.8 cm TL; 95% CI: 52.3–65.3; Figure 6B) or uterine development (59.0 cm TL, 95% CI: 53.4–64.7; Figure 6C). Pregnant females ranged from 55.0 to 84.0 cm TL and had a TLP_{50} estimated at 63.7 cm TL (95% CI: 58.9–68.4; Figure 6D). Maternal females ranged from 55.0 to 81.0 cm TL, and the TLM_{50} was estimated at 66.2 cm TL (95% CI: 61.9–70.5; Figure 6E).

Ovarian and Uterine Fecundity

All females analyzed had ovarian follicles in both ovaries, varying in number from 1 to 69 per female (mean ± SD, 23.6 ± 15.8; mode = 14) and between 1 and 46 follicles per ovary (right: 12.8 ± 8.3, mode = 10; left: 13.6 ± 8.8, mode = 8). There were no significant differences in the number of ovarian follicles between the left and right ovaries (Z = 0.90, df = 151, P = 0.365).

There were significant differences among number of ovarian follicles by size-groups (KW test: $H_{2,\ 168}$ = 64.63, $P <$ 0.0001). The small group presented the highest mean and maximum number of ovarian follicles (16.8 and 62,

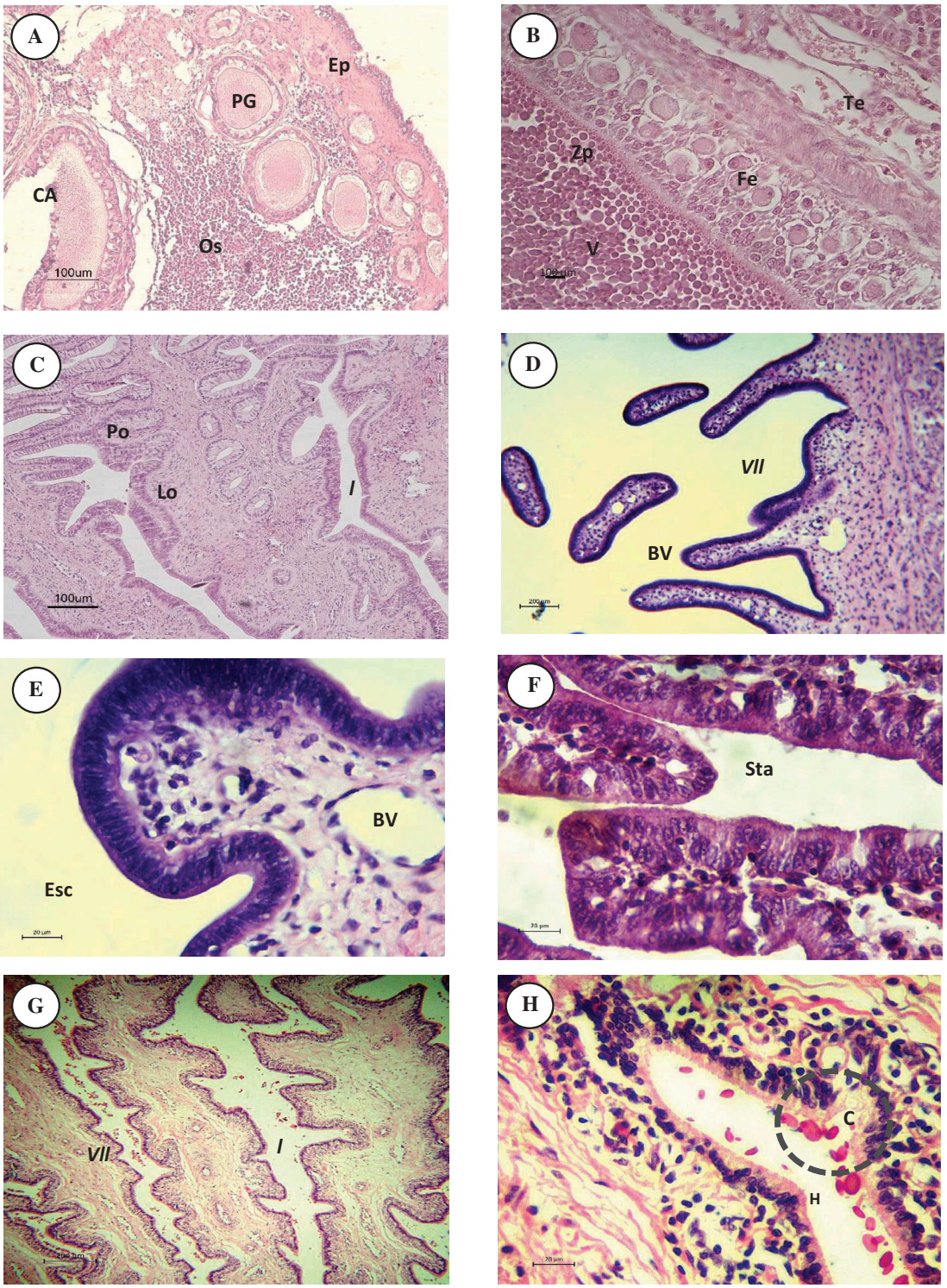

FIGURE 5. Micrographs of reproductive structures of female Giant Electric Ray. (**A**) Transverse section of an ovary in the immature developing phase (ovarian index 2). (**B**) Transverse section of a vitellogenic oocyte from a female in the ovulation-capable phase (ovarian index 3). (**C**) Transverse section of the anterior oviduct of a female in the ovulation-capable phase with oocytes in the uterus. (**D**) Transverse section of the uterus of an immature developing female (uterine index 2). (**E**) Transverse section of the uterine villi from a female in the immature developing phase (uterine index 2). (**F**) Transverse section of the uterus from a female in the mature-not-pregnant phase (uterine index 3). (**G**) Transverse section of the uterus from a female with late-stage embryos (uterine index 4E). (**H**) Longitudinal section of the uterine villi from a pregnant female with late-stage embryos (uterine index 4E) and secretory crypts (C, i.e., within dotted line). Abbreviations are as follows: Ep = peritoneal epithelium, CA = cortical alveolar oocytes, PG = primary growth oocytes, Os = ovarian stroma, Zp = zona pelucida, Fe = follicular epithelium, Te = theca externa, V = granules of vitellogenin, Lo = oviductal lobules, l = lumen, Po = oviductal plates, Vll = uterine villi, BV = blood vessel, Esc = simple cylindrical epithelium, Sta = stratified cylindrical epithelium, and H = histotroph.

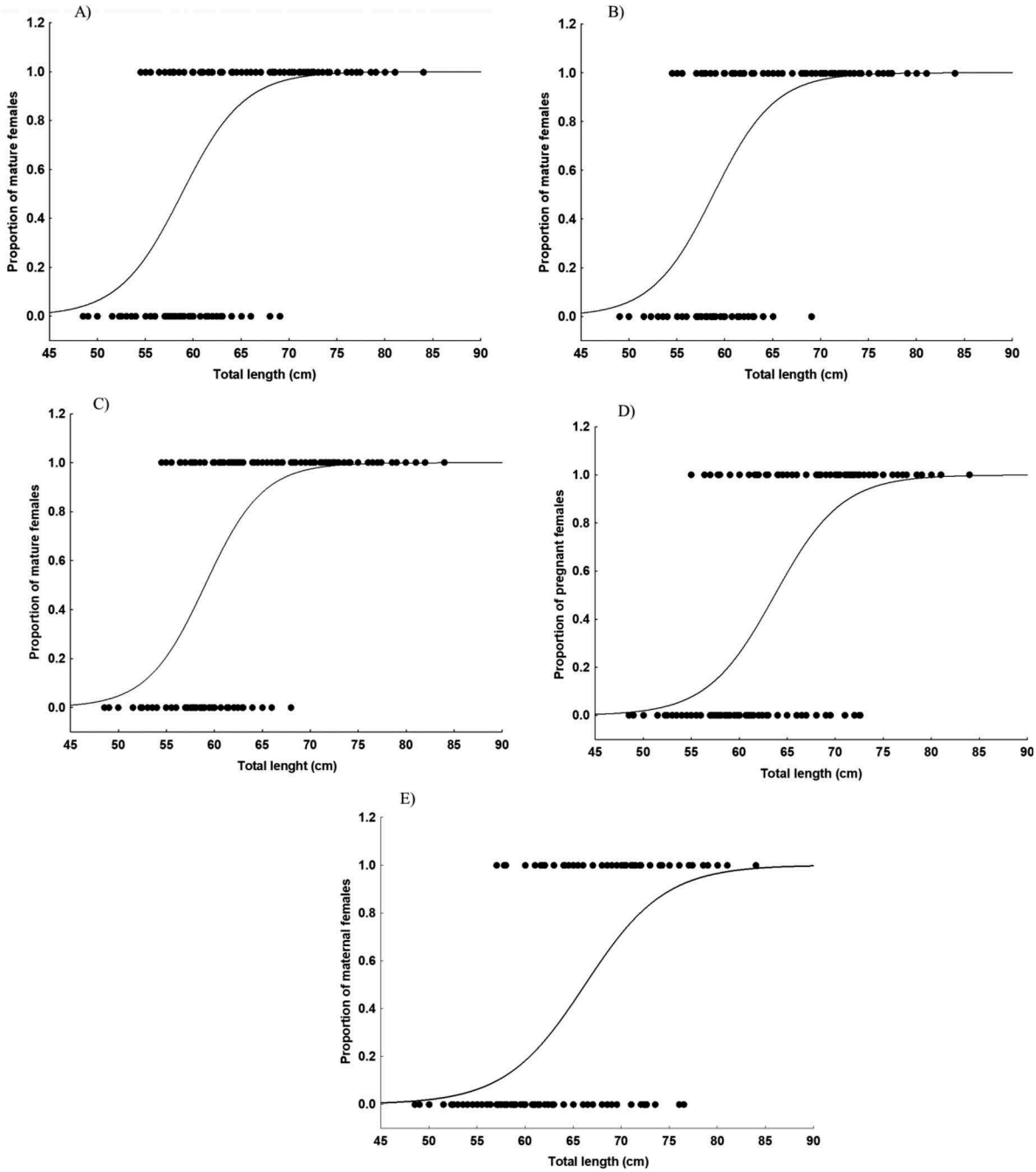

FIGURE 6. Maturity ogives for female Giant Electric Rays in relation to TL (cm). (**A**) Ogive by maturity phase. (**B**) Ogive based on ovary condition. (**C**) Ogive based on uterus condition. (**D**) Pregnancy ogive. (**E**) Maternity ogive.

respectively), the medium group presented a mean of 4.7 and maximum of 26, and the large group presented a mean of 7 and maximum of 17 ovarian follicles (Figure 7A). The total number of ovarian follicles showed a clear relationship with female size; however, only females that were ≥57 cm TL had more than 50 follicles of different sizes, and medium and large follicles were present in size-group ≥ 52.5 cm TL (Figure 7A). There was a significant difference between the ovarian index and the total number of ovarian follicles (KW test: $H_{2,\ 168}$ = 12.796, P = 0.0017). The ovarian fecundity (mean = 7.0, max = 17) had no relationship with the size of the female (r^2 = 0.0180, P = 0.464), but only females ≥ 58 cm TL had 10 or more follicles capable of being ovulated (Figure 7A).

A total of 45 females had embryos in the uterus, and sizes ranged from 0.1 to 14.5 cm TL (n = 307; 123 females, 88 males, and 96 undefined). The embryo female : male sex ratio

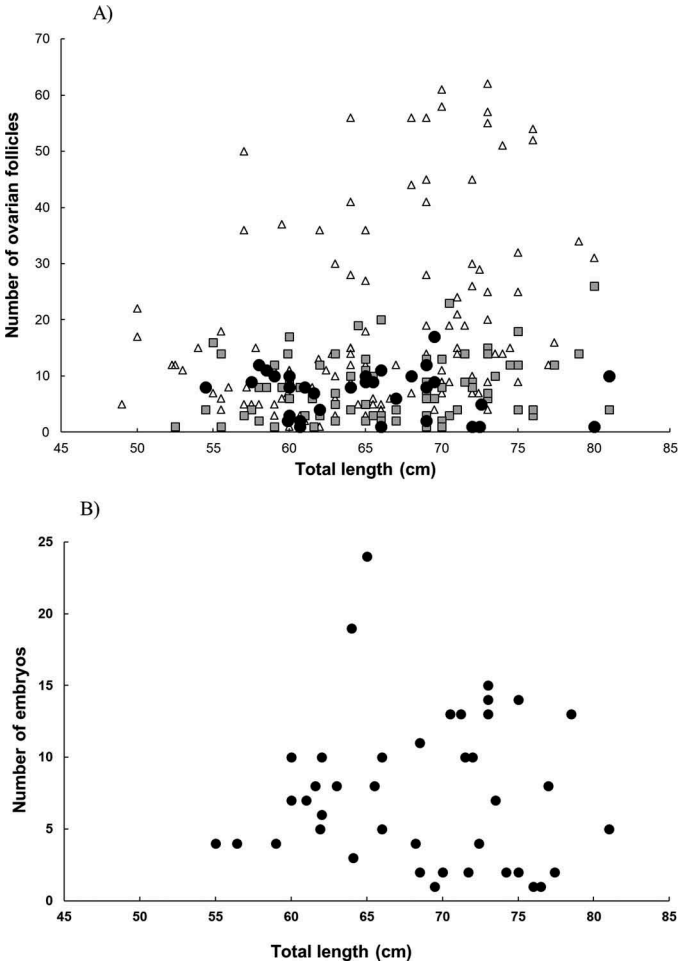

FIGURE 7. Fecundity relationships in female Giant Electric Ray. (**A**) Total length (cm) and number of ovarian follicles by group (triangle = small, square = medium, and circle = large) and (**B**) TL (cm) and number of embryos by female.

was 1.3:1, which was not significantly different from the expected 1:1 ratio ($\chi^2 = 5.805$, $P = 0.984$). Embryonic fecundity varied between 1 and 24 embryos (6.6 ± 5.3, mode = 2), and the number of embryos did not vary significantly between left and right uteri ($Z = 0.95$, df = 34, $P = 0.431$). There was no relationship between uterine fecundity and female TL ($r^2 = 0.0009$, $P = 0.850$, $n = 41$; Figure 7B), but only females ≥ 60 cm TL had more than seven embryos and females ≥ 70.5 cm TL had 13 or more embryos. The highest fecundity observed, 24 embryos, was in a 75-cm-TL female.

Reproductive Cycle

Maximum ovarian follicle lengths varied significantly among months (KW test: $H_{11,\ 180} = 87.12$, $P < 0.0001$), and the largest ovarian follicles occurred in July, August, and September (31.5, 29.3, and 31.8 cm, respectively; Figure 8A). Follicular growth and development begins in May and ends (ovulation events) from August to September. Additionally, September was the only month in which oocytes were found in the anterior oviduct and large postovulatory follicles were seen in the ovary, evidence of recent ovulation. However, two females had large ovarian follicles in February (19.8 cm) and April (20.5 cm).

The median size of embryos varied significantly across months in which they were present (KW test: $H_{8,\ 307} = 249.84$, $P < 0.0001$; Figure 7B). Additionally, eggs were observed in the uterus during all months, which can be indicative of embryonic diapause. There were two periods of embryonic growth: from October to January–February and from May to August. This later period corresponded to the season in which the majority of females were captured. Embryonic size at birth was between 12.4 and 14.5 cm TL. There was a clear tendency of synchronous development between vitellogenesis and gestation; females with embryos in the two earliest stages of development (Formation and Early in Figure 8C) also had small ovarian follicles, while females with embryos in the Late developing stage had the largest ovarian follicle lengths (Figure 8C).

The male GSI did not vary significantly among months (KW test: $H_{7,\ 38} = 4.93$, $P = 0.667$), although GSI was highest in October (2.94). Female GSI did vary significantly among months (KW test: $H_{11,\ 160} = 64.60$, $P < 0.0001$), and the highest mean value occurred in August. Two homogeneous subsets were observed in female GSI; GSI values in May, July, and August significantly higher than those in January, February, October, and December (Bonferroni adjusted $P < 0.004$; Table 3). The GSI began to decrease in October and remained low until April, suggesting little ovarian growth during these months.

Finally, when considering the percentage of females in each reproductive phase throughout the year (Table 4), there is one peak of ovulation between July and September (highest percentage of ovulation-capable females) but two peaks of parturition (presence of Late developing embryos; Table 4). During the first, primary peak in parturition, the females enter a period of embryonic diapause from October through April; embryo development reactivates in May and birth occurs between August and September. During the second, minor peak in parturition, gestation begins in October, embryonic diapause does not occur, and females give birth in January to February.

DISCUSSION

This study provides evidence that the Giant Electric Ray has a continuous annual reproductive cycle; one peak of ovulation occurs between July and September but two peaks of parturition occur (minor peak in January–February and

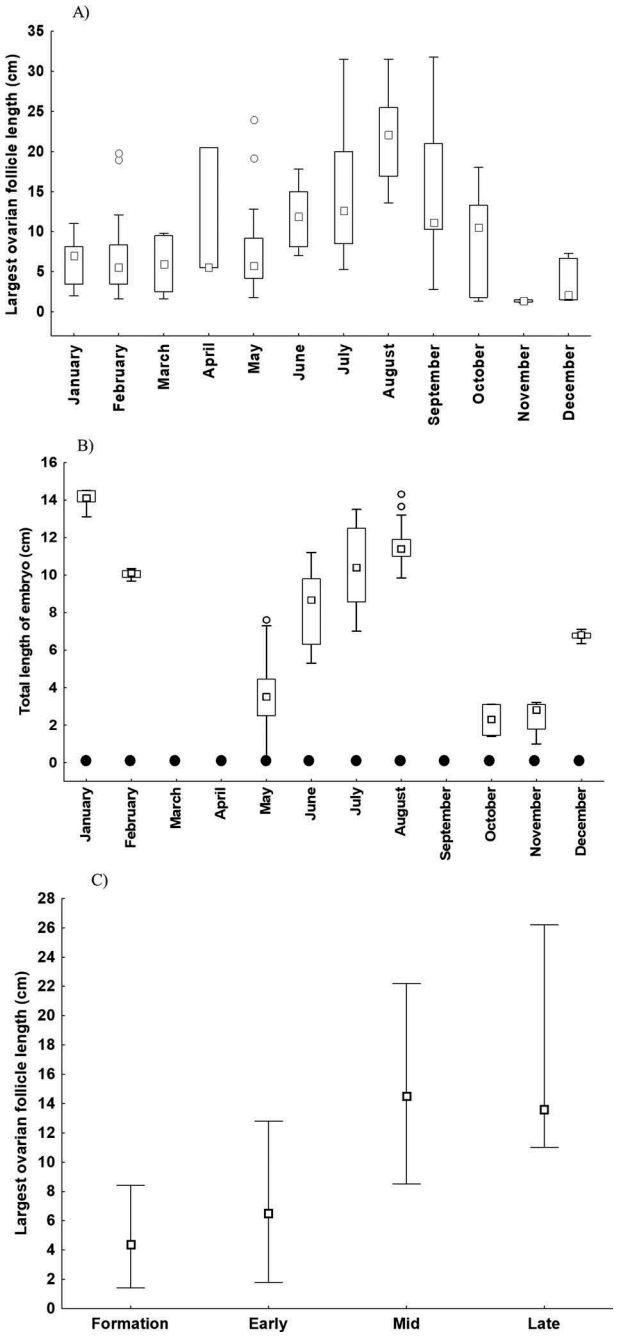

FIGURE 8. Relationships of lengths of ovarian follicles and embryos in Giant Electric Ray. (**A**) Monthly variation of largest ovarian follicle length in mature females and (**B**) Monthly variation of intra-uterine embryo TL (filled circles indicate eggs in the uterus). The lower and upper boundaries of the boxes indicate the 25th and 75th percentiles, respectively, while the square (□) inside the boxes indicates the medians. Whiskers indicate the nonoutlier range and circles indicate outliers. (**C**) Relationship between largest ovarian follicle length and embryo developmental stage. Whiskers indicate the minimum and maximum length while the square (□) indicates the medians.

major peak in August–September). These two peaks of births suggest that a majority of female Giant Electric Rays undergo embryonic diapause, as previously suggested by C. J. Villavicencio-Garayzar, M. E. Mariano, and C. H. Downtonn (abstract presented at the 6th Indo-Pacific Fish Conference, 2001) for this species, and similar to reports for other species of rays (Lessa 1982; Simpfendorfer 1992; Morris 1999; Waltrick et al. 2012). Additionally, in contrast to previous reports of matrotrophy in this species (Villavicencio-Garayzar 2000), histological evidence of secretory material in endometrial tissue during late pregnancy suggests the Giant Electric Ray presents limited histotrophy as a reproductive mode.

The largest sizes of Giant Electric Rays examined in this study are smaller to those reported by Villavicencio-Garayzar (2000) for the Bahía Almejas, Baja California Sur, Mexico (females = 93 cm TL, males = 67 cm TL) and those reported for the Ecuadorian Pacific Ocean by J. J. Palma-Chávez, A. F. Romero-Caicedo, J. E. Pincay-Espinoza, and M. Carrera-Fernández (abstract presented at the 6th National Symposium of Sharks and Rays, 2014) (females = 110 cm TL, males = 83 cm TL), which could mean different populations were sampled. In contrast, it is possible that the sizes recorded for the present study are smaller than previously reported because the rays live in more protected areas (which function as mating or nursery habitats) within a gulf or that the method of capture did not allow collection of larger individuals.

Although the observed sizes in our study were smaller than those found in previous studies, they correspond primarily to sexually mature individuals, similar to that found in previous studies. Thus, the effect of fishing gear selectivity is likely not a concern when comparing studies. Smaller sized organisms, such as neonates and juveniles, are likely to inhabit protected areas, such as shallower waters and marshes, as also suggested by Villavicencio-Garayzar (2000) for Bahia Almejas. However, since artisanal fishers only target large-sized organisms, we were not able to collect neonates during this study. Neonates are likely located in areas that are not accessible for fishing with gill nets, or they may leave the bay immediately after parturition. This is supported by Rudloe (1989) observation that Brazilian Electric Rays *N. brasiliensis* in the Gulf of Mexico tend to move to shallower areas during warm seasons and retreat to deeper areas during the cold seasons for parturition, which would also explain the absence of neonates in our study.

The greater proportion of female Giant Electric Rays relative to males in the Bahía de La Paz has also been reported for this species in Bahía Almejas (Villavicencio-Garayzar 2000). A possible explanation for this pattern is that males only enter the shallow, protected waters of the bays for mating, as the months when they were most abundant (July–September) is the mating season (Villavicencio-Garayzar, Mariano, and Downtonn, abstract), which coincides with the months of greatest follicular length and highest GSI in females. Other species of Narcinidae also show female-dominated sex ratios; Brazilian Electric Rays on the coast of São Paulo, southeastern Brazil, had a female : male sex ratio of 2.2:1, a result

TABLE 3. Mean monthly values (±SE) of the gonadosomatic index in female Giant Electric Rays. Similar letters indicate homogeneous subsets (Tukey nonparametric post hoc test with Bonferroni adjustment, $P < 0.004$). n = sample size.

Month	n	Mean GSI ± SE
January	13	0.79 ± 0.245 z
February	33	0.532 ± 0.106 z
March	7	0.503 ± 0.124 zy
April	4	0.341 ± 0.102 zy
May	24	0.953 ± 0.378 y
June	3	0.625 ± 0.088 zy
July	22	1.53 ± 0.259 y
August	19	3.384 ± 0.39 y
September	6	2.773 ± 0.882 zy
October	10	0.525 ± 0.076 z
November	4	0.432 ± 0.034 zy
December	15	0.455 ± 0.05 z

attributed to fishing gear selectivity (Rolim et al. 2015). Female Lesser Electric Rays in Santa Marta, Colombia, also have a sex ratio of 2.4 females per male (Moreno et al. 2010). In general, sexual segregation is a common characteristic among diverse species of elasmobranchs and has been attributed to differences in sizes between sexes in order to reduce predation or differences in feeding grounds, although there are not sufficient studies to support these hypotheses (Wearmouth and Sims 2010).

This is the first study to microscopically describe testicular development in the Giant Electric Ray. We observed different spermatogenic phases such as those previously described in mature elasmobranchs (Maruska et al. 1996). Giant Electric Ray testes have multiple germinal zones, similar to previous histological descriptions in batoids (Pratt 1988), and the mature spermatocysts were generally concentrated in the periphery of the testes near the efferent ducts, as described by Hamlett (1999) for batoid species.

Villavicencio-Garayzar (2000) defined maturity in male Giant Electric Rays in Bahía Almejas as the presence of semen in the vas deferens; that author did not evaluate the different stages of spermatogenesis as we did in this study. Here, we defined functional maturity based on histological examination of the testes and seminal vesicles as well as the presence of spermatozoa in the claspers. Another criterion to define maturity in male elasmobranchs is the degree of calcification of the clasper (Abdel-Aziz 1994); only mature individuals have claspers that are completely calcified, as was found in this study for all males examined.

In mature male Giant Electric Rays, the relationship between clasper length and fish size (TL) seems to have a linear tendency, similar to reports by Villavicencio-Garayzar (2000) in Bahía Almejas for the Giant Electric Ray and for the Brazilian Electric Ray studied by Rolim et al. (2015). It is likely that this relationship has a inflection point; however, we cannot define the type of growth in relation to the clasper and TL since we do not have immature individuals. The absence of juveniles has also been reported in other zones and in the

TABLE 4. Percentage of maturity phases throughout the year in female Giant Electric Ray.

		Immature (%)	Mature (%)						
			Pregnant						Not pregnant
Month	Total number of females	Developing	Ovulation capable or actively ovulating (with eggs in the uterus)	Eggs in the uterus[a]	Embryos in formation	Early embryos	Mid embryos	Late embryos	Regressing
January	18	16.7	0.0	66.7	0.0	5.6	0.0	0.0	11.1
February	35	14.3	11.4	54.3	0.0	0.0	2.9	0.0	17.1
March	10	20.0	0.0	80.0	0.0	0.0	0.0	0.0	0.0
April	11	18.2	27.3	54.5	0.0	0.0	0.0	0.0	0.0
May	33	24.2	15.2	15.2	24.2	15.2	3.0	0.0	3.0
June	13	23.1	0.0	15.4	15.4	15.4	23.1	0.0	7.7
July	31	41.9	32.3	3.2	0.0	3.2	6.5	12.9	0.0
August	35	14.3	54.3	2.9	0.0	0.0	8.6	17.1	2.9
September	14	28.6	42.9	28.6	0.0	0.0	0.0	0.0	0.0
October	23	13.0	30.4	34.8	4.3	4.3	4.3	0.0	8.7
November	8	37.5	0.0	25.0	12.5	12.5	0.0	0.0	12.5
December	29	72.4	0.0	20.7	0.0	0.0	3.4	0.0	3.4

[a]Females with eggs in the uterus, but with ovary index = 4.

Mexican Pacific Ocean for the same species (Villavicencio-Garayzar 2000; Rolim et al. 2015).

Females of various species of elasmobranchs have varying sizes of reproductive structures, including some organs that are dysfunctional (Dodd 1972; Castro et al. 1988). However, in female Giant Electric Rays, both ovaries are of similar length and both contain fertile ovarian follicles. The weights of the left and right ovaries were different, but this may have been an artifact of field sampling since the largest ovaries tended to break and expel their oocytes and ovarian stroma prior to obtaining measurements in the laboratory. The presence of two functional ovaries and uteri of equal size has been previously reported for the same species in Bahía Almejas (Villavicencio-Garayzar 2000) as well as for Ocellated Torpedo (Capapé et al. 2000), Pacific Electric Ray (Neer and Cailliet 2001), and Brazilian Electric Ray (Rolim et al. 2015), and is thus likely a common feature among electric rays.

The elongated ovarian follicles of the Giant Electric Ray is unique among batoid species, and it was described previously by Villavicencio-Garayzar (2000) for this species. This form of the ovarian follicles may be related to uterine space as a reproductive tactic whereby the female can provide a lot of yolk to the embryos in a reduced space. Moreno et al. (2010) reported Lesser Electric Ray oocytes as "yellowish threads," which likely corresponds to the elongated form present in Giant Electric Ray, although those authors did not mention the length of the oocytes.

All females evaluated for this study had ovarian follicles in different stages of follicular development, results that are very different than those reported by Villavicencio-Garayzar (2000) for the same species in Bahía Almejas where females < 61 cm had no gametogenic activity. However, Villavicencio-Garayzar (2000) did report ovarian follicles of greater length (to 50 cm) than those encountered in the present study. This may be due to females being larger (up to 93 cm TL) in Bahía Almejas than in the present study. The presence of ovarian follicles ≤ 15.9 cm in length in completely developed ovaries (ovary index = 4) suggests a continuous production of ovarian follicles throughout the year, which further suggests the species has continuous reproductive activity (Koob and Callard 1999). In addition, the presence of postovulatory follicles in the ovary in September suggests recent ovulation or a period of preovulation, which is common in elasmobranchs (Lutton et al. 2005).

To define the birth size of the Giant Electric Ray in Bahía Almejas, Villavicencio-Garayzar (2000) used the largest size of embryos in the uterus that had small yolk sacs as well as the size of the smallest neonate captured (15.7 cm TL) and defined a size of birth from 14 to 16 cm TL, which corresponds to 14.3–16.4% of the asymptotic size in his study. Unfortunately, we did not capture any neonates in Bahía de La Paz, so the size at birth was based on the largest embryo in utero without a yolk sac, following morphological characteristics proposed by Braccini et al. (2007) and evidence from Moreno et al. (2010) for the Lesser Electric Ray. We defined a birth size between 12.4 and 14.5 cm TL, which, although smaller than the defined birth size from rays in Bahía Almejas, corresponds to 14–16.4% of the asymptotic size of the sampled population, similar to the asymptotic size reported by Villavicencio-Garayzar (2000). Interestingly, we did not find a difference in the sex ratio of embryos, similar to reports by Villavicencio-Garayzar (2000) for Bahía Almejas. These observations support the idea that adults and juveniles are temporally and spatially segregated by sex, rather than there being a preponderance of females in the population.

Villavicencio-Garayzar (2000) defined the Giant Electric Ray as a matrotrophic species and considered that one-third of the weight of the embryo depended on the mother through uterine milk obtained through the uterine villi; however, this investigator did not carry out studies that defined the percentage of yolk consumed, as proposed by Guallart and Vicent (2001). In our study, we did not observe uterine milk, and the material secreted by the uterine villi observed in histological sections was only a few droplet granules located near the glandular crypts. Additionally, the muscular tissue and serosa layer of the uterus were very thin, while in elasmobranchs with a dependence on uterine milk these structures are very thick (Colonello et al. 2013). Thus, the characteristics observed in the present study suggests that Giant Electric Ray is likely a species with limited histotrophy (matrotrophic), based on the secretions from secretory crypts in the endometrium and the increased vascularization in that tissue, since according to Moura et al. (2011) this is evidence of a certain type of nutrition secreted by the mother. Additionally, these secretory crypts are composed of more than six layers of cells that make up the glandular tissue, in contrast to the related Ocellated Torpedo that has only one to two cell layers in the uterine villi and a viviparous reproductive type with vitelline sac and no histotrophy (Ranzi 1934; Hamlett et al. 2005).

Histotrophy can be used as supplemental food when the embryo has used up the yolk sac (Hamlett et al. 2005). However, histochemical analyses are necessary to define the type of secretion and to determine if it is a nutrient substance secreted by the mother to provide embryonic nutrition, since the difference between limited histotrophy and lecitotrophy is very subtle (Huveneers et al. 2011). Alternatively, a comparison of the dry weights of eggs and embryos at term, as proposed by Guallart and Vicent (2001), could also help determine the extent of matrotrophy exhibited by Giant Electric Rays.

The median size at maturity estimated for the species in Bahía de La Paz represents 66.2% of the estimated maximum asymptotic length (88.4 cm TL), which is smaller than that proposed by Villavicencio-Garayzar (2000) for this species in Bahía Almejas (62–63 cm TL; 68–69%). This difference is likely due to the catch sizes for this study (maximum TL = 84 cm), which were smaller than those from Bahía Almejas

(Villavicencio-Garayzar 2000). However, in both studies, the mature population is >60% of the asymptotic size. In contrast, other species of Torpediniformes, such as Lesser Electric Ray and Pacific Electric Ray reach maturity at 53.5% and 53%, respectively, of their estimated asymptotic length (Neer and Cailliet 2001; Moreno et al. 2010). It should be noted that the median size at maturity based on the condition of the ovaries was similar to the median size at maturity estimated considering all reproductive structures together. Thus, when monitoring the species, the condition of the ovaries (mature or immature) can be used to define the maturity of the organism. This is the first study to evaluate the median size at maternity for the Giant Electric Ray, which represents 74.9% of estimated asymptotic length. This suggests that only the largest females in the population of Bahía de La Paz contribute to recruitment the following year if they are not captured.

The total number of ovarian follicles encountered for Giant Electric Rays (69) was similar to that reported for Pacific Electric Rays (Neer and Cailliet 2001) and Variable Torpedo Rays *T. sinusperisici* (Shrikanya and Sujatha 2014) of 55 oocytes for both species. However, ovarian fecundity based only on large vitellogenic ovarian follicles is less than uterine fecundity in Giant Electric Ray. It is likely that the ovarian fecundity of the Giant Electric Ray is underestimated, since the ovarian follicles could have been damaged or expelled during the manipulation of specimens in field.

The uterine fecundity of Giant Electric Rays in Bahía de La Paz (1–24 embryos) was slightly greater than that reported for this species in Bahía Almejas (4–20 embryos: Villavicencio-Garayzar 2000). This difference could be due to an underestimation in the Bahía Almejas population as pregnant females could have aborted their embryos during capture, since specimens were recovered from fishing gear after several hours. In our study, we identified a female with a contracted cervix and a fecundity of 24 embryos, suggesting she did not abort any embryos, which provides support for our estimations. Lower fecundities have been observed in other Torpediniformes, such as Lesser Electric Ray (1–14, Moreno et al. 2010), Pacific Electric Ray (17, Neer and Cailliet 2001), and Marbled Electric Ray *T. marmorata* (3–16, Consalvo et al. 2007), although all these species are smaller than the Giant Electric Ray and thus can be expected to have lower fecundities. In addition, in some species the number of embryos is related to embryo size, such as in Ocellated Torpedo, which has a fecundity of 28 small embryos (12.5 cm TL, Capapé et al. 2000).

In contrast to Villavicencio-Garayzar (2000) and Villavicencio-Garayzar, Mariano, and Downtonn (abstract) who only found ovarian follicles from May to August in Giant Electric Rays from Bahía Almejas, we found females with ovarian follicles year-round. However, ovarian follicle growth began in May and follicles achieved their greatest lengths in September, although previtellogenic follicles were present throughout the year. It is possible that previous studies did not report the presence of previtellogenic follicles in the ovary.

The presence of oocytes descending into the anterior oviducts during September in our study was also reported for this species in Bahía Almejas (Villavicencio-Garayzar 2000). Females with ovarian follicles ≥ 16 cm were not observed in October in rays in either study, indicating that ovulation and mating end in September, which may be closely related to the increase in temperature in the summer months in both bays. While only two females were recorded with follicles near ovulation size (19.8 and 20.5 cm) in February and April, respectively, it is likely that these follicles are atypical since both females contained only one follicle of this size and all others were ≤15.9 cm in length.

Gestation and vitellogenesis occurred synchronously in Giant Electric Rays in both Bahía de La Paz and in Bahía Almejas (Villavicencio-Garayzar 2000; Villavicencio-Garayzar, Mariano, and Downtonn, abstract). Furthermore, gametogenic development was observed in females in all months, and this is mainly because once the mother gives birth, she is ready to ovulate immediately after parturition suggesting this is a species with a continuous annual reproductive cycle (Koob and Callard 1999). However, this was not the case for a single female in January with not only late developing embryos but also with ovarian follicles < 16 cm, too small for ovulation. Likely, this small (66 cm TL) female was reproducing for the first time and did not go through embryonic diapause like all the other females in the population. This atypical behavior could be a physiological response to the environment or a respite from the continuous reproductive periods; this possibility can only be corroborated with hormonal analysis as discussed by Lopes et al. (2004) and Murphy (2012).

January and August represent the two periods of parturition, although we only observed one female in January ready to give birth, and another in February with embryos in the mid-developing stage. Thus, it appears there are two peaks of birth, following two separate paths of development. The majority of the females fertilized in summer (August–September) pass through a period of embryonic diapause from October until April and have fertilized eggs in the uterus during this time. Fertilized eggs can remain in the blastodisc stage from 4 to 10 months (Simpfendorfer 1992; Morris 1999). Later, embryo development is reactivated in May and parturition occurs in August and September. In the second possible pathway without embryonic diapause, as seen in a minority of females in Bahía de La Paz, embryonic development of fertilized oocytes begins immediately in October and parturition occurs in January–February. The period of embryo development is the same in both pathways (5 months), but one group of the population delays embryo development for 7 months during the coldest time of the year, followed by activation of the embryo development period in the summer

months. According to Wyffels (2009), it is common to identify an embryonic diapause period in females with fertilized eggs in the uterus but without visible embryos for long periods of time, and this is also common in populations with synchronous reproductive cycles, as we observed for Giant Electric Rays in Bahía de La Paz.

Embryonic diapause was suggested previously for the Giant Electric Ray by Villavicencio-Garayzar (2000) and Villavicencio-Garayzar, Mariano, and Downtonn (abstract) in Bahía Almejas. Furthermore, other species of rays have also been reported to have embryonic diapause, such as Bluntnose Stingray *Dasyatis say* (Simpfendorfer 1992; Morris 1999), Brazilian Guitarfish *Rhinobatos horkelii* (Lessa 1982), and Whiptail Stingray *D. brevis* and Shovelnose Guitarfish *R. productus* (Villavicencio-Garayzar, Mariano, and Downtonn, abstract).

This study reports previously unknown reproductive data for the Giant Electric Ray, an important commercial species in Bahía La Paz, Mexico. Of particular concern is that most of the mature individuals caught in the area are pregnant females with eggs or at different stages of gestation. Furthermore, the principal months of birth are July to September, yet elasmobranch fishing closures in Mexico only occur from May 1 to July 31 (DOF 2012). Our data suggest female Giant Electric Rays are vulnerable to capture during the primary birthing months, which may negatively impact population recruitment and jeopardize the population's recovery from overharvesting. Although the Mexican law is meant to protect different species of elasmobranchs, the complexity of incorporating biological information with fishing and resource dynamics is challenging, particularly when biological information is lacking. Thus, information provided here is important for the evaluation of the population of Giant Electric Rays in Bahía de La Paz and should be considered when future policies and management plans are drafted to protect this species.

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