

**REPRODUCTIVE BIOLOGY OF THE VENEZUELA  
ROUND STINGRAY *UROTRYGON VENEZUELAE*  
SCHULTZ FROM THE COLOMBIAN CARIBBEAN**  
**Biología reproductiva de la raya redonda de Venezuela *Urotrygon*  
*venezuelae* Schultz del Caribe colombiano**

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**ABSTRACT**

As for most batoid species, little is known about the basic biology of the Venezuela round stingray *Urotrygon venezuelae* (Urotrygonidae). This study presents information about the reproductive biology of the species, including fecundity, embryonic development stage, relationship between maternal size and fecundity, gonadosomatic (GSI) and hepatosomatic (HSI) indices, sex ratios, maturity size and size at birth. With all this information, a preliminary reproductive cycle is proposed. A total of 269 specimens were caught with beach seine in Salguero beach, Colombian Caribbean Sea, between August 2005 and October 2006. We propose for *U. venezuelae* a biological cycle with three reproductive peaks: November-December, March-April and August. Size at sexual maturity was calculated in 176 mm (total length) for females and 227 mm for males; fecundity ranged between one and six embryos per female. We found that cloacal diameter and liver weight were better predictors for fecundity than total length for *U. venezuelae*.

**Key words.** Urotrygonidae, bioecology, Batoids, Colombia, Caribbean sea.

**RESUMEN**

Como en muchas especies de batoideos, hay una carencia de conocimiento acerca de la biología básica de la raya redonda de Venezuela *Urotrygon venezuelae*

(Urotrygonidae). Este estudio presenta información sobre la biología reproductiva de la especie, incluyendo fecundidad, estado de desarrollo embrionario, relación entre la talla materna, fecundidad, índice gonadosomático (IGS) y hepatosomático (IHS), proporción sexual, talla de madurez y talla de nacimiento. Con esta información se propuso de manera preliminar un ciclo reproductivo para la especie, el cual debe ser corroborado con estudios posteriores. En total se capturaron 269 especímenes empleando chinchorro playero en playa Salguero, Caribe colombiano, entre agosto de 2005 y octubre de 2006. Se propone para *U. venezuelae* un ciclo biológico conformado por tres picos reproductivos: noviembre-diciembre, marzo-abril y agosto. La talla de madurez sexual para las hembras se calculó en 176 mm (longitud total) y 227 mm para los machos y la fecundidad se encontró fluctuando entre uno y seis embriones por hembra. Se propone que el diámetro cloacal y el peso del hígado fueron mejores predictores de fecundidad que la longitud total para *U. venezuelae*.

**Palabras clave.** Urotrygonidae, bioecología, Batoideos, Colombia, Mar Caribe.

## INTRODUCTION

*Urotrygon venezuelae* Schultz 1949, belongs to the American endemic family Urotrygonidae, which has only two genera with 16 species (Nelson 2006). There is very little information available on this species. It is of conservation concern by IUNC assessed as Near Threatened (Kyne & Valenti 2007) due to its restricted distribution to western Venezuela inshore coastal waters and because this species is most probably taken as bycatch in artisanal and commercial trawl fisheries, although no species-specific data are available (Valdez & Aguilera 1987, Charlier 2000, Mendoza *et al.* 2003).

This species, as all the ones included in the family Urotrygonidae are placental viviparous with a yolk sac and trophonemata (Hamlett *et al.* 2005). Studies in thorny round stingray *Urotrygon chilensis* and dwarf round stingray *Urotrygon nana*, show low fecundity and short gestation periods (Guzmán 2006, Rubio 2009). The basic biological information available about its size at sexual maturity, fecundity, reproductive cycle, longevity, fishery records and morphometric characteristics for *U. venezuelae* are based only on few specimens and in most of the cases is scarce (Acero *et*

*al.* 2008, Bigelow & Schroeder 1953, Dahl 1971, Valdez & Aguilera 1987).

In the Colombian Caribbean Sea, fishermen using beach seines target small and medium pelagic fish of commercial importance such as Clupeidae, Carangidae and Trichiuridae. Some commercially valuable batoid species, such as *Dasyatis guttata* and *Dasyatis americana*, are also caught. Other batoids as *Rhinobatos percellens*, *Narcine bancroftii* and *U. venezuelae*, are part of bycatch but do not have any use or market value. *U. venezuelae* was reported as a coastal species captured by artisanal fishermen with beach seines in Salguero beach at depths between 5 to 10 m (Téllez *et al.* 2006). The present study provides information about the reproductive biology of *U. venezuelae*, including fecundity, embryonic development stage, relationship between maternal size and number of embryos, reproductive period, sex ratios, size at sexual maturity and size at birth.

## MATERIALS AND METHODS

**Study area.** Salguero beach is located toward south of Santa Marta, Colombian Caribbean (11°10'N - 74°13'W and 11°11' N - 74°14'W). During the dry seasons

(major summer: August–September; minor summer: December–January), the Colombian Caribbean is affected by the Caribbean current and the upwelling of deep waters, respectively (Andrade *et al.* 2003, Franco 2005).

**Sampling.** Specimens were captured with a fishing gear called beach seines during 45 minutes hauls, effectuated daily (five days per week) each month between August 2005 and October of 2006, although no samples were collected during January. Total individuals number (abundance) captured per haul was standardized per month and hour. For each specimen total length (TL), disc width (DW), disc length (DL) and cloaca diameter (CD maximum measure of the urogenital opening in females) were measured, in addition to gutted weight (GW) and liver weight (LW). Reproductive conditions were evaluated using macroscopic observations (Table 1). Fecundity was estimated as the total number of embryos per female (Conrath 2005). For each embryo, total length was measured as well as its location inside the uteri. Developmental stage of embryos were determined using the

size of each embryo compared to maximum size reported for the species (362 mm present study), according to its size embryos were classified as follows (modified from Liu *et al.* 1999): early (0-10%), midterm (10.1-20%) and late embryos (20.1-33%). Birth size was calculated based on smallest free swimming juvenile (Conrath 2005).

**Data analysis.** Results from the Gonadosomatic Index (GSI), Hepatosomatic Index (HSI) and Fulton’s conditional factor (K), were considered in order to propose breeding season. Sex ratio was tested for a significant departure from the expected 1:1 using the chi-square test with a confidence level of 95%.

Size at sexual maturity ( $l_{50\%}$ ) was modeled by fitting the logistic function of mature proportion with 10 mm TL size interval; the curve was fitted by applying non-linear regression technique (King 2007).

$$P(l) = \frac{1}{1 + \exp(a + b * l)}$$

**Table 1.** Maturity scale based on the observed characteristics for *U. venezuelae* (Snelson *et al.* 1988).

Females	Immature I	Aqueous and small ovary, poor development and thin oviducts.
	In maturation II	Thin ovary, uniform appearance without oocytes, some development oviducts.
	Without embryos IIIa	Ovary with visible oocytes, thick oviducts and a partially spread uteri.
	Mature With embryos IIIb	Ovary with oocytes, Widened uteri with embryos, occupied more than a half of the visceral cavity, walls with villus (trophonemata) and histotrophe in the entire uterus.
	After giving birth IV	Widened uteri with bloody, flaccid and villus walls.
Males	Immature I	Aqueous and small testis, genital ducts poor development, small and flaccid claspers, rhipidion close, no seminal fluids.
	In maturation II	Ventral surface of the testis lobed, dorsal surface smooth, genital ducts differentiated, ductus deferens forming rolls at least half-way of the epididymis, Larger and partially calcified claspers.
	Mature III	Lobed testis, epididymis cross the visceral cavity, the ductus deferens joining the testis and the seminal vesicles, claspers calcified rhipidion open and abundant seminal fluids.

Where  $P(l)$  is the mature proportion,  $a$  and  $b$  are the parameters estimated,  $l$  the size interval. The size at 50% maturity is  $l_{50\%} = a/b$  (King, 2007).

Size maturity was calculated choosing mature females (stages IIIa, IIIb, and IV); in the case of males only the mature ones were included (stage III) (White *et al.* 2001). Because the assumption of homogeneity of variances was not fulfilled, the non-parametric Wilcoxon test was improved to establish statistically significant differences between right and left ovary weights (White *et al.* 2001). Relationships between total length (TL) and number of embryos (NE) were evaluated using linear regression (Conrath 2005). Additionally, Generalized Additive Models (GAM) (Hastie & Tibshirani 1990) were used in order to find the maximum embryos output related to other biological predictors such as TL, DL, DW, CD, GW, K, HI, GSI and LW, to follow a biological perspective, since the youngest and the oldest specimens are not functionally reproductive (Koops *et al.* 2004). GAM is a modern non-parametric statistic tool which allow to fit models according to ecological theory (Katsanevakis & Maravelias, 2009). An additive model is an extension of linear models, allowing that linear functions of the response variable (fecundity) and the predictors could be replaced by smooth functions and do not require functional assumptions (Agenbag *et al.* 2003). These models are expressed as:

$$y = \alpha + \sum_{i=1}^n f_i(X_i) + \varepsilon$$

where  $y$  is the response variable (fecundity),  $X_i$  is the predictor,  $\alpha$  is a constant, and  $\varepsilon$  is the error;  $f_i$  was estimated with a spline (s) smooth, as well as a Gaussian family. The GAM diagnostic procedure included the significance value (P) and the percentage of deviance explained of the model. The deviance is analogous to the variance and

the null deviance is analogous to the total variance. Therefore, the null deviance minus residual deviance is the variance explained by the model.

$$\text{Explained deviance} = \frac{\text{Null deviance-residual deviance}}{\text{Null deviance}}$$

Additionally, the Akaike information criterion (AIC), which is a measure of model deviance corrected for the number of predictors, was used, and the model with lowest AIC was chosen (Burnham & Anderson 2002).

## RESULTS

A total of 269 specimens were collected (138 females and 131 males) between August 2005 and October 2006. A mean of 4.5 ( $\pm$  7.3) individuals/hour was estimated for sampled months. Highest values were registered during September (26.7 ind/h), November (7.5), May (5.3) and June (4.5). Lowest abundance was reported for April (0.3) and March (0.5), no samples were analyzed during January (2006).

Female size ranged from 101 mm to 362 mm TL, while males ranged from 102 to 295 mm TL. Left ovaries from mature females were larger and heavier than right ones (T=2045, N= 35, P=0.1). A total of 35 gravid females (stage IIIb) were reported in eight of twelve sampled months (Table 2). Fecundities for these females were calculated from 1 to 6 embryos for both uteri. Embryo size fluctuates from 7.5 to 118.0 mm, representing 25.3%-36.4% of maternal size.

Development scale for *U. venezuelae* embryos includes an early stage from 7.5 to 36 mm, 0-10% of maximum female TL (362 mm), characterized by an oval shape disc, loopy spiracles, absence of spine and translucent body color. Midterm stage includes embryos between 37 to 72 mm, 10.1 to 20.0% maximum TL, disc similar in shape to adults, rudimentary spine and loopy spiracles. Late stage size from 73 to 118 mm, 20.1 to 33.0% maximum TL,

characterized by adults coloration, developed spine and spiracles. A total of 54 and 20 embryos were found in left and right uteri, respectively. Temporal progression between embryonic development degrees was not detected; in fact, it was possible to observe embryos from different developmental stages during the same month (Table 2).

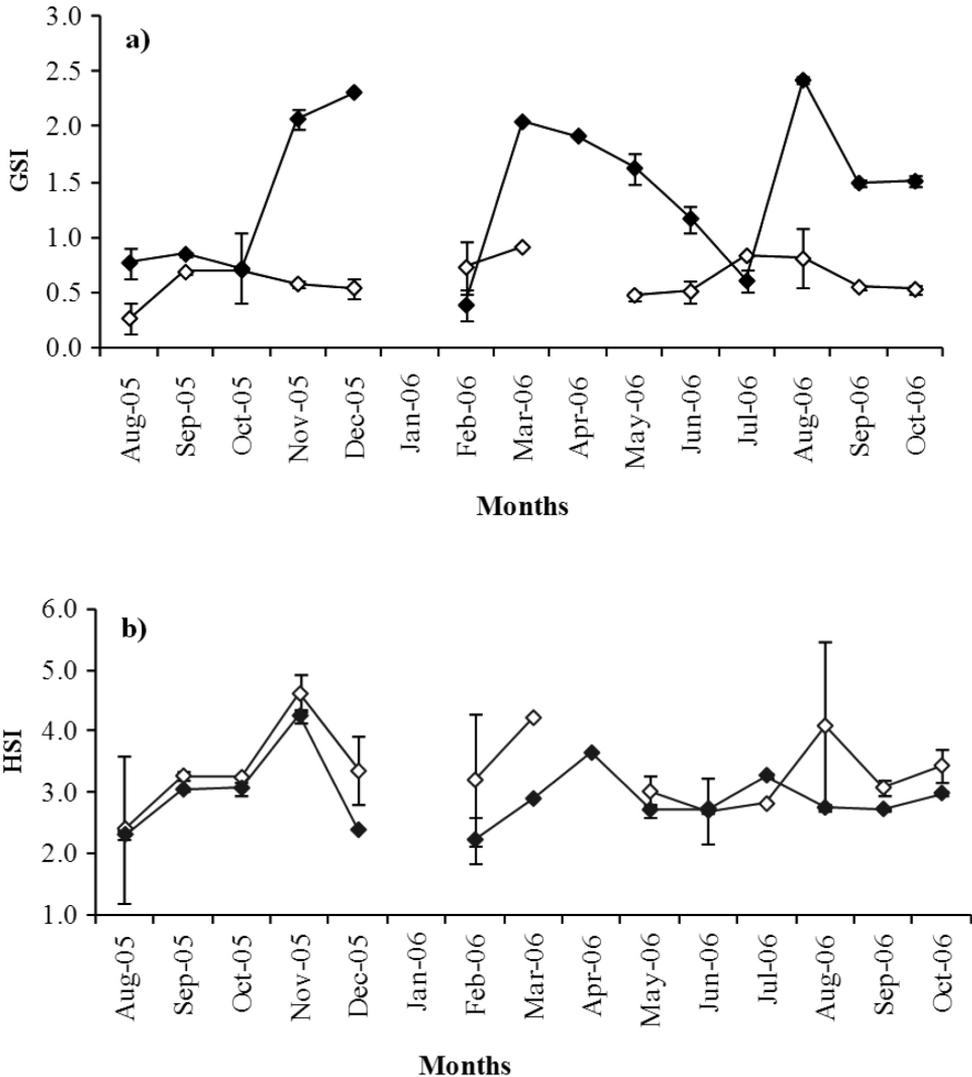
Breeding size from embryo was previously calculated in 114 mm TL and 58 mm DW, nevertheless smallest free swimming individuals were captured, in that sense birth size for *U. venezuelae* for the Colombian Sea is proposed in 101 mm TL.

**Table 2.** Gravid females of *U. venezuelae*, number of embryos and total length interval. Catch from August 2005 to October 2006.

Embryos development state	Month	Number of gravid females	Number of embryos
Early	Sept-05	1	1
	Nov-05	2	2
	Dic-05	0	0
	Feb-06	0	0
	May-06	1	1
	Ago-06	0	0
	Sep-06	1	1
	Oct-06	2	3
Medium	Sept-05	3	8
	Nov-05	3	7
	Dic-05	0	0
	Feb-06	1	2
	May-06	0	0
	Ago-06	1	2
	Sep-06	4	10
	Oct-06	2	3
Late	Sept-05	0	0
	Nov-05	3	8
	Dic-05	1	1
	Feb-06	2	4
	May-06	4	14
	Ago-06	2	5
	Sep-06	1	1
	Oct-06	1	1

GSI shows a highest value in August (2006) ( $2.42 \pm 0.03$ ) and the lowest during February ( $0.38 \pm 0.13$ ) (Fig. 1a). Three reproductive peaks were observed, the first from November ( $2.07 \pm 0.09$ ) to December (2.13), a second from March to April and the third peak during August (Fig. 1a). HI were highest in November ( $4.25 \pm 0.11$ ), April ( $4.18 \pm 0.14$ ) and August 2006 ( $3.06 \pm 0.11$ ), the lowest values appears in February ( $2.21 \pm 0.37$ ) (Fig. 1b). Breeding may occur during November and May, when the maximum number of late stage embryos with larger sizes were founded. According to the cited results it's difficult to determine the duration of the gestational period, nevertheless it could occur between the three cited peaks, each one lasting about three or four months. There were no differences in sex ratio ( $p$ -value = 0.216) between 138 females and 131 males collected, in that sense sex ratio for *U. venezuelae* was 1:1 in this locality.

Size at sexual ( $l_{50\%}$ ) was 176 mm TL for females (96 mm DW,  $r^2=0.99$ ) and 227 mm TL for males (119 mm DW) ( $r^2=0.99$ ) (Fig. 2). Linear regression between fecundity (number of embryos) and TL showed a low correlation coefficient (Fig. 3). In that sense there is a weak but visible tendency of females within 250 y 325 mm TL may produce a higher number of embryos, in spite of some of them can also contain only one. This non-linear relationship was modeled using GAM (Fig. 4). The cloacal diameter (CD) and liver weight (LW) showed a significant relationship and higher explained variance of fecundity (Table 3). Higher fecundities are related to CD from 10.8 to 13.5 mm when four or more embryos were produced, also in individuals from 7.0 to 11.5 g LW and 150 mm DL. Fecundity shows a strong increment between 160 and 200 g of gutted weight (GW). The HI between 5 and 7 shows high fecundity values (embryos), but K did not describe a relationship with fecundity, showing the lowest explained deviance (Table 3).

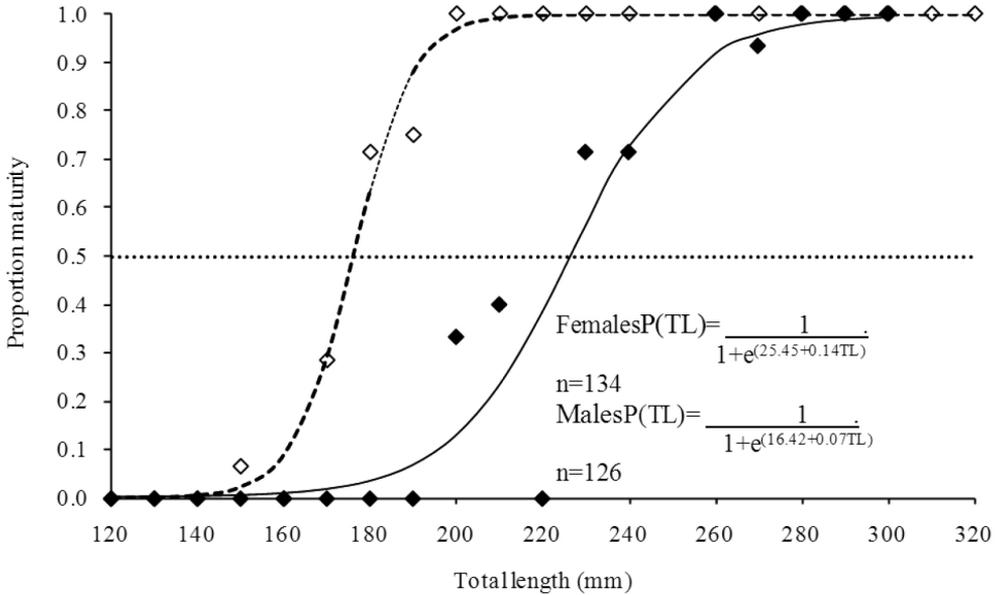


**Figure 1.** Variation of a) Gonadosomatic index (GSI) in males (◆) and females (◇), b) Hepatosomatic index (HSI) of males (◆) and females (◇) of *U. venezuelae* in Salguero beach between August of 2005 and October of 2006 (mean and Standard Error).

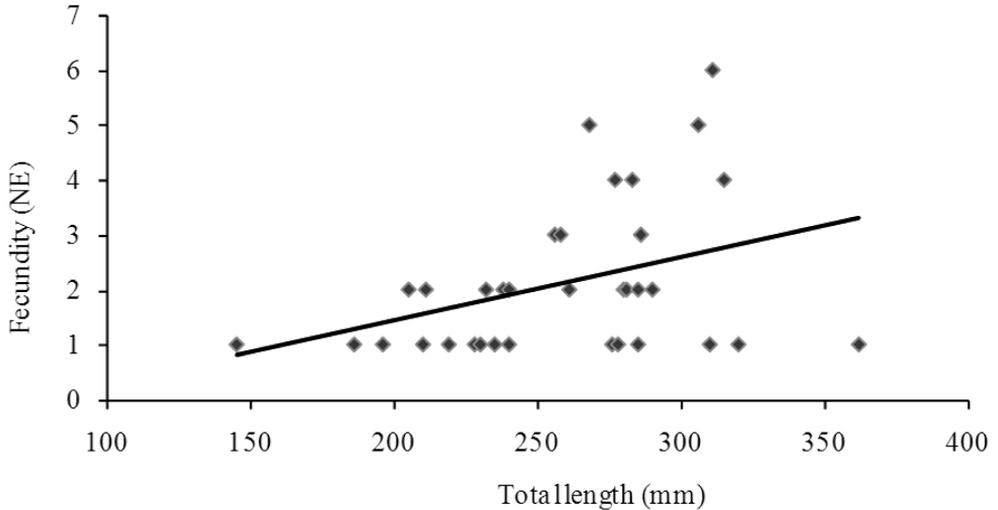
**DISCUSSION**

McEachran & Carvalho (2002) restricted *U. venezuelae* to Venezuela Gulf, but the species is also present in the Colombian Caribbean Sea (Dahl 1971, Téllez *et al.* 2006; Mejía-Falla *et al.* 2007). No catch per unit effort (CPUE) has been reported for this species

in the area. As was described for *Urotrygon rogersi* (Mejía-Falla *et al.* 2012), females of *U. venezuelae* reached greater maximum TL than males. Maximum size of female (362 mm TL) was larger than 290 mm reported by McEachran & Carvalho (2002) for the species.



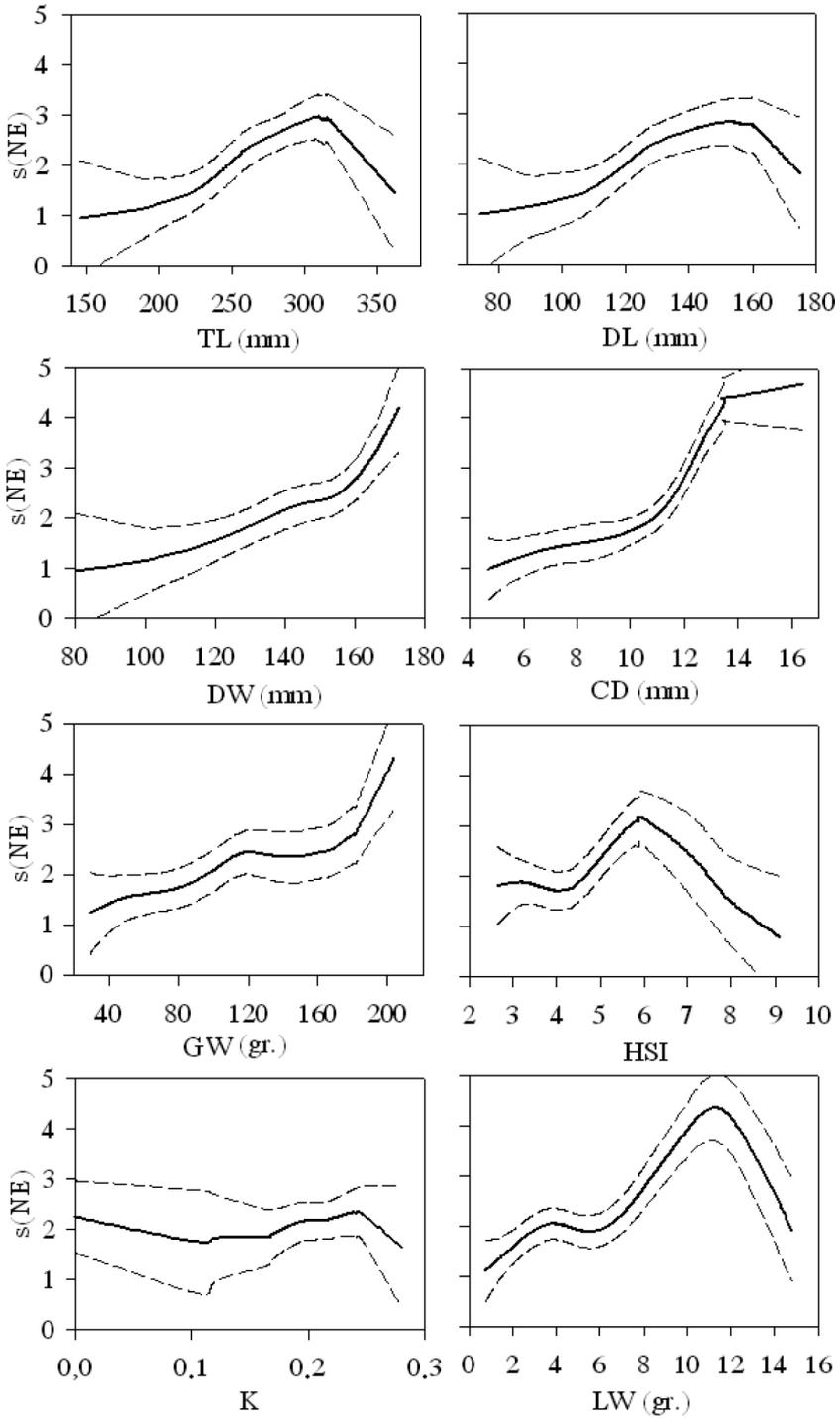
**Figure 2.** Size at 50% maturity ( $L_{50\%}$ ) for female (◇ dotted line) (176 mm TL) and male (◆ continuous line) (227 mm TL) of *U. venezuelae*.



**Figure 3.** Relationship between fecundity (number of embryos) and total length (TL) of *U. venezuelae* in Playa Beach. The curve was fitted by  $\text{Fecundity NE} = 0.0115\text{TL} - 0.8482$  ( $r^2 = 0.1371$ ).

For the Venezuela round stingray, both ovaries were present in adults and also in juveniles, this contrast with was described in *Urobatis halleri*, for which both ovaries were observed only in immature fish (Babel 1967). Embryos of *U. venezuelae* were registered in both uteri

as was mentioned for *U. rogersi* (Mejía-Falla *et al.* 2012). In contrast embryos of *Urolophus lobatus* (White *et al.* 2001) and *Gymnura micrura* (Kobelkowsky 2004), were only present in left uterus.



**Figure 4.** GAM modeling results of functional relationships between fecundity (number of embryos NE) and predictors of *U. venezuelae*.

**Table 3.** GAM modeling results between the variable fecundity (NE) and the predicted variables, explained deviance by the model, p-value and AIC criteria. (TL: total length, DL: disc length, DW: disc width, CD: cloaca diameter, GW: gutted weight, Hepatosomatic Index (HSI), Fulton's conditional factor (K) and LW: liver weight).

Characteristic	Exp.Dev.(%)	Df	p-value	AIC
TL	26.69	33	0.19	56.00
DL	21.06	34	0.38	63.45
DW	28.49	34	0.41	58.73
CD	<b>57.93</b>	33	<b>0.01</b>	<b>43.35</b>
GW	28.61	30	0.24	67.07
HI	23.82	30	0.07	69.79
K	4.80	33	0.69	77.10
LW	<b>41.30</b>	34	<b>0.01</b>	<b>50.60</b>

Most of the females were categorized as immature (I), as was previously described for Salguero beach (Téllez *et al.* 2006); however, mature males (III) were the most important during the present study differing from the mentioned authors who described higher abundance of immature males. Differences could be explained because Téllez *et al.* (2006) analyzed fish from February to October 2004 and main rainy season was not sampled. Sexual maturity size in males was lower than size proposed by Dahl (1971) (300 mm TL) and Téllez *et al.* (2006) ( $282.3 \pm 0.98$  mm TL) for the Caribbean Sea of Colombia. Estimations of  $l_{50\%}$  for *U. venezuelae* females in 96 mm DW, were similar to those cited for *U. rogersi* considering uterus width (11.8 cm), ovary (12 cm) and oviducal gland condition (12.3 cm) (Mejía-Falla *et al.* 2012). Size at sexual maturity in *U. venezuelae* was lower in females than in males, backing the hypothesis proposed by Braccini & Chiaramonte (2002) for some small sized skates of the family Rajidae, where females reach size of sexual maturity before males, *Psammobatis extenta* and *U. venezuelae* are good examples of this. In large size species, such as *Dipturus*

*chilensis* sexual maturity size is lower in males than females (Quiroz *et al.* 2009).

Development stage and size of embryos were similar among both uteri, opposite to *U. halleri* where embryos of the right uteri had bigger sizes than those from the left one (Babel 1967). Gravid females of *U. venezuelae* showed mature embryos simultaneously with viable oocytes inside the ovaries as was described for *U. lobatus* (White *et al.* 2001). In this sense, vitellogenesis occurs while gestation (Fahy *et al.*, 2007), pregnant females become fertilized and new embryos continue developing in upper uterus. Some authors (Wenbin & Shuyuan 1993) proposed that in Myliobatiformes all embryos inside a female grow at the same rate, however *U. rogersi* (Mejía-Falla *et al.* 2012) and *U. venezuelae* (present study) showed different size embryos. Despite its small size, *U. venezuelae* produces a large number of embryos (up to 6) compared to the larger *Urolophus paucimaculatus* (White & Potter 2005), *Trygonoptera personata* and *Trygonoptera mucosa* (1-2 embryos) (White *et al.* 2002), *U. rogersi* (1-3 embryos) (Mejía-Falla *et al.* 2012) and similar to *U. halleri* (1-6 embryos) (Babel 1967), *Urotrygon microphthalmum* (3-6 embryos) (Almeida *et al.* 2000), *Urolophus jamaicensis* (1-5 embryos) and *U. lobatus* (2-6 embryos) (Fahy *et al.* 2007).

Intrauterine embryos size in *U. venezuelae* fluctuated between 7.5 to 11.4 cm TL, similar to embryos from *U. rogersi* which reported a minimum size of 3.9 and maximum 14.7 cm (Mejía-Falla *et al.* 2012). Abortion post capture was registered in 10 gravid females, showing embryos tails outside the cloaca, which may lead to an underestimation of fecundity. This event has been observed in other small stingrays such as *U. lobatus* (White *et al.* 2001) and *U. rogersi* (Mejía-Falla *et al.* 2012). Short gestation periods from two to four months are been proposed

for some urolophids (Hamlett & Koob 1999). Nevertheless is not possible to identify the gestation period for *U. venezuelae* due to low number of pregnant females. Evidences about reproductive strategies such as embryonic diapauses described for *T. personata* (White *et al.* 2002) and *R. percellens* (Grijalba-Bendeck *et al.* 2008) must be evaluated for the Venezuela round stingray.

Based on GSI, HI, gonadal condition and embryo presence and development, it is possible to propose a reproductive cycle of three to four months for *U. venezuelae* in Salguero beach, with three reproductive peaks (November-December, March-April, and August). Copulation events occurs between July and October. Mature males and gravid females stay close to the coast during gestation process, until the occurrence of parturition from May to November, when rainy season may increase the quantity of available food. The described pattern was similar to the one proposed for *U. rogersi*, in the eastern Colombian Pacific ocean, where a triannual reproductive cycle is proposed, occurring intrauterine growth during April, August and December, parturition possibly during April to May, August to September and December to January; a 4-5 months gestation period was proposed for this species (Mejía-Falla *et al.* 2012). Short gestation periods are been described for aplacental viviparous batoids of the families Urolophidae and Urotrygonidae (Wourms 1977; Hamlett & Koob, 1999).

The relationship between fecundity and TL for *U. venezuelae* registered a low  $r^2$ , showing that TL is a weak predictor of fecundity (Conrath 2005). Its opposite to the positive relationship proposed between litter size and maternal size for *U. rogersi*, females  $\geq 12.5$  disc width had more than two embryos and those  $\geq 16.0$  cm had more than three (Mejía-Falla *et al.* 2012). It seems that greater female size increases the space to lodge a greater number of embryos (Babel 1967), which has been observed in

sharks, such as *Galeorhinus galeus* (Peres & Vooren 1991). The main reason may be that youngest and oldest specimens are less productive, the young because they have not reached maturity size and oldest because their reproductive potential could be decreased.

Other authors have attempted to relate fecundity with length (Villavicencio-Garayzar 1995, Fahy *et al.* 2007, Quiroz *et al.* 2009), but they have not kept in mind other factors that could be more important for fecundity, such as cloaca diameter, liver weight and even weight. Mejía-Falla *et al.* (2012) reported a linear relationship between DW and fecundity as was described by Hleap *et al.* (2009), nevertheless relationship between breeding size and maternal morphometric must be consider in further studies.

The analysis of maternal condition has different ways to be understood, in this sense some authors recommended a selection criteria based on a systematic search through all possible ways to find the best model that could give statistical significance, like the Akaike's Information Criterion that we used in this study (Koops *et al.* 2004).

We found that cloacal diameter and liver weight were the best fecundity predictors. When mature females are young (150 to 230 mm TL) their pups are smaller than the pups of larger females (250 to 320 mm TL). As a consequence, younger females have less muscular distension according to embryo size, while in bigger females cloaca diameter increase with pup size. Liver weigh was a good predictor of fecundity, when the value of this index reaches 10 - 12 g, females seems to contain more pups. It seems that for *U. venezuelae* it is more important to be in good condition, in order to assure offspring higher fitness, the latter must be confirmed by assessing a major number of gravid females. On the other hand Fulton's K and available energy are correlated mainly in bony fishes

(Koops *et al.* 2004), but in elasmobranchs K values and energy seem to be unrelated. Therefore, it seems that K is not a good predictor to explain maternal condition in rays. Hence, liver weight could represent stored available energy for fecundity period, because rays, like other elasmobranchs, store lipids in the liver that is the responsible of maintaining maternal fitness during active reproduction (Kebir *et al.* 2007, Néchet *et al.* 2007). Liver weight also reflects food supply that is influenced by seasonal patterns and environmental conditions.

Results of this study suggested that Salguero beach represents an important breeding and growing area for the Venezuela round stingray *U. venezuelae*. Nevertheless, deeper knowledge about this endemic species is needed. Additional biological and ecological aspects must be considered as demographic and stock assessment in conjunction to environmental conditions are required; all these information can support propose management measures for the species conservation in this area of the Caribbean Sea.

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