Reproductive biology of the lesser guitarfish *Zapteryx brevirostris* from the south-western Atlantic Ocean

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This study provides the first detailed information on the reproductive biology of the lesser guitarfish *Zapteryx brevirostris* in the south-western Atlantic Ocean between latitudes 34° and 42° S. A total of 320 males (196–647 mm total length, L_T) and 336 females (200–640 mm L_T) were analysed. No significant differences were observed in size at 50% maturity between males (499 mm L_T) and females (506 mm L_T). Three seasonal patterns were related to the testes mass, the development of spermatocysts and the presence of spermatozoa in genital ducts. Females showed a 3 year reproductive cycle, with 2 years of ovarian maturation and 1 year of gestation, including a mean annual fecundity of one to two embryos. Synchronicity of both ovulating females and males with sperm in genital ducts indicated that ovulation and hence mating occurs during winter. Birth was assumed to occur during winter, but neither ovulation nor mating is simultaneous with this reproductive event. Despite its low economic value, a decline in abundance in the region, together with a low reproductive efficiency, indicates that the *Z. brevirostris* population should be monitored closely.   © 2011 The Authors

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Key words: elasmobranch; maturity; ovary; reproduction; seasonality; spermatogenesis.

INTRODUCTION

In the south-western Atlantic Ocean, the Rhinobatiformes are represented by the chola guitarfish *Rhinobatos percellens* (Walbaum), the Brazilian guitarfish *Rhinobatos horkelli* Müller & Henle and the lesser guitarfish *Zapteryx brevirostris* (Müller & Henle) (Menni & Stehmann, 2000; Cousseau *et al*., 2007). *Rhinobatos horkelii* and *Z. brevirostris* are sympatric species reported from Brazil (São Paulo, latitude 23° S) to northern Argentina (Claromecó, latitude 39° S) (Menni & Stehmann, 2000; Vooren *et al*., 2005; Cousseau *et al*., 2007).

Rhinobatids, like other Chondrichthyes, exhibit slow growth, late sexual maturity and low fecundity (Lessa *et al*., 1986; Abdel-Aziz *et al*., 1993; Capapé & Zaouali,

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1994; Villavicencio-Garayzar, 1995; Kyne & Bennett, 2002; Seck *et al.*, 2004; Ismen *et al.*, 2007; Márquez-Farías, 2007; Blanco Parra *et al.*, 2009), which makes them vulnerable to overexploitation (Dulvy *et al.*, 2000, 2003; Dulvy & Reynolds, 2002).

In Brazilian waters, rhinobatids are landed as by-catch by artisanal and shrimp-trawling fisheries (Lessa *et al.*, 1999; Vooren & Klippel, 2005). Stock assessment in southern Brazil shows that commercial catch per unit effort of *R. horkelii* decreased *c*. 85% (Vooren *et al.*, 2005), but no information is available for *Z. brevirostris*. These batoids are also captured south of latitude 34° S as part of the multispecies coastal fishery between latitudes 34° and 42° S, below 50 m deep and they are entirely discarded (Massa *et al.*, 2004; Tamini *et al.*, 2006; Domingo *et al.*, 2008). In this region, the abundance of guitarfishes was reduced by nearly 50% between 1994 and 1999 (Massa *et al.*, 2004).

Biological knowledge of *Z. brevirostris* from the Brazilian coastal region, between latitudes 23° and 26° S, includes some aspects of the population structure (Santos *et al.*, 2006), sexual development (Batista, 1991; Ponz Louro, 1995) and fecundity and embryo development (Batista, 1991; Abilhoa *et al.*, 2007). This species attains a maximum total length (L_T) of 527 mm (Santos *et al.*, 2006; Abilhoa *et al.*, 2007) and matures at 420–430 mm L_T (Batista, 1991; Ponz Louro, 1995). South of latitude 34° S, there is information about morphology (Castello, 1971; Cousseau *et al.*, 2007) and feeding habits (Barbini *et al.*, 2011). The maximum L_T recorded for fish from the region is 650 mm.

An assessment of the reproductive biology of a species is central to understanding its ecology and to developing effective conservation measures. This study analyses the reproductive biology of *Z. brevirostris* in the coastal south-western Atlantic Ocean between latitudes 34° and 42° S because the biological variables of *Z. brevirostris* appear to be different from those estimated for Brazilian waters.

MATERIALS AND METHODS

Zapteryx brevirostris were collected between 2003 and 2007 from bottom trawl surveys carried out by the R.V. '*Dr. Eduardo L. Holmberg*' and R.V. '*Cap. Oca Balda*' of the Instituto Nacional de Investigación y Desarrollo Pesquero (National Institute for Fisheries Research and Development), Argentina. The survey area included the coastal (<50 m depth) south-western Atlantic Ocean between latitudes 34° and 42° S (Fig. 1). Cruises were conducted in May 2003, December 2003, July 2004, November 2005, February 2006 and June 2006. The gear used was a standard Engel-type bottom trawl of 120 mm inner mesh-size, with a vertical height of 5 m and a horizontal opening of 20 m. The standard tow duration was 15 min at a speed of 7·408 km h^{-1} (4 knots). Additionally, samples of testes were collected by on-board commercial fishery observers during 2007.

The L_T (mm), total body mass (M_T, g), liver mass (M_L, g) and sex of each specimen were recorded. To evaluate differences in the L_T and M_T relationships between sexes, linear regression coefficients were calculated and compared using ANCOVA. The M_T and M_L relationships were also compared to determine possible differences between males and females.

In males, clasper length and mass of paired testes (M_{GT}) were recorded. The external clasper length was measured from the tip of the pelvic fin to the tip of the clasper (L_{EC}, mm), and the inner clasper length from the end of the cloaca to the tip of the claspers (L_{IC}, mm). In females, uterus (W_U, mm) and oviducal gland width (W_{OG}, mm), ovarian follicle diameter (mm) and ovary mass (M_{GO}, g) were recorded. Maturity status was assessed by visual inspection of the reproductive organs following Capapé & Zaouali (1994). Three and six reproductive stages were determined for males and females, respectively (Table I). Changes in reproductive organs (claspers, testes, ovaries, oviducal glands and uteri) were plotted
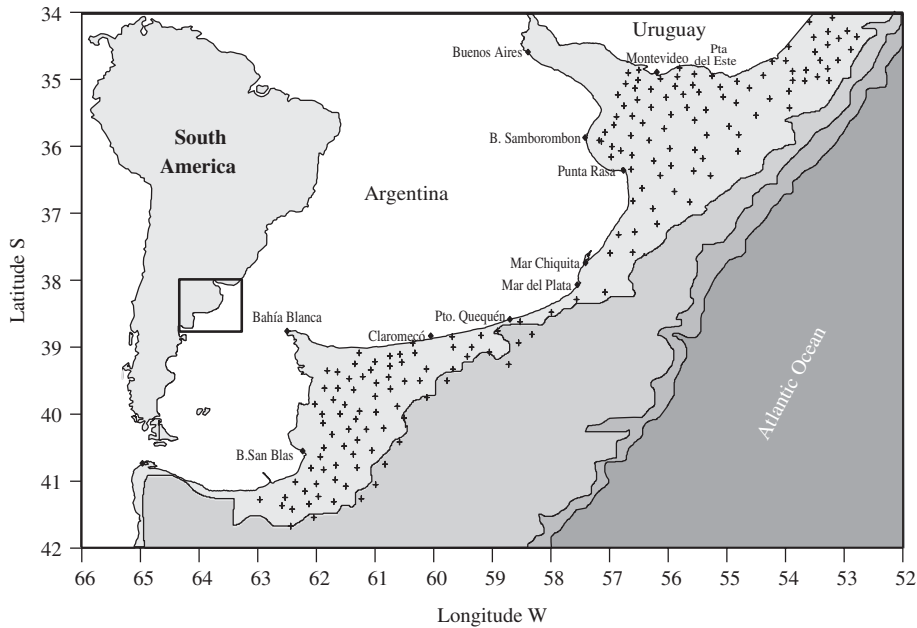
FIG. 1. Map of study area and trawl locations (+).

against L_T to further assess the onset of maturity. Logistic models were used to assess the proportion of mature males and females at any L_T (stage 3; Table I), and for females the proportion at maturity and maternity (Walker, 2005) condition (stages 4, 5 and 6; Table I); the maximum proportion of individuals in maternal condition was adjusted with parturition frequency (Walker, 2005). Model parameters and the ogive with the 95% CI were estimated by the maximum likelihood method (Walker, 2005) using R statistical software, version 2.11.1 (R Development Core Team; www.r-project.org). Differences in the L_T at which 50% were mature (L_{T50}) between both sexes were evaluated using a log-likelihood ratio test.

The timing of the male reproductive cycle was determined macroscopically using monthly variations of the gonado-somatic index (I_G, where $I_G = 100\ M_{GT}\ M_T^{-1}$) and the presence of spermatozoa in the deferent ducts. Histological assays of testes and genital ducts to verify macroscopic assessment were conducted. For this purpose, samples for histology were removed and fixed in 10% formalin. In the laboratory, these samples were placed in tissue cassettes, dehydrated and embedded in paraffin. A $1-3$ μm section was stained with standard haematoxylin and eosin. Prepared slides were examined and classified into spermatogenic stages based on Maruska *et al*. (1996). The number of spermatocysts in each stage was measured across representative full lobe sections of the testis. The mean proportion of the testis occupied by each stage in different months of the year was compared to determine seasonal variation in testis development. On the basis of published histological studies on elasmobranch testes (Maruska *et al*., 1996; Conrath & Musick, 2002; Kyne & Bennett, 2002; Sulikowsky *et al*., 2004, 2005; Yamaguchi & Kume, 2009), three stages were distinguished: immature (spermatocytes I and II), maturing (spermatids) and mature (after spermatogenesis).

To assess the female reproductive cycle, temporal changes in ovarian condition were evaluated by examining the diameter of the largest ovarian follicle (D_{LOF}) and the mean ovary mass [gonado-somatic index (I_G), where $I_G = 100\ M_{GO}\ M_T^{-1}$]. Egg capsules and size of the embryos *in uteri* collected throughout the year were compared to further define the reproductive cycle.

The hepato-somatic index (I_H) was calculated from $I_H = 100\ M_L\ M_T^{-1}$. An ANOVA followed by Tukey's *post hoc* test (Zar, 1984) was used to determine whether there were significant differences in monthly variation of reproductive variables (*e.g.* I_G and D_{LOF}).

TABLE I. Criteria used to determine the stages of maturity of male and female *Zapteryx brevirostris*

		Males	Females
Juvenile	Stage 1	Testes not developed, with small lobules and abundant epigonal organ. Short claspers not extending beyond the posterior edge of pelvic fins	Small ovaries lacking differentiation of follicles, oviducal glands undifferentiated from uteri, which are slender and straight
	Stage 2	Maturing testes with differentiated lobules, although surrounded by abundant epigonal organ. Straight genital ducts. Elongated claspers, lacking calcification	Small ovaries with some ovarian follicles differentiation lacking mature oocytes; uteri narrow and constricted
Mature	Stage 3	Mature testes with lobules fully developed. Convoluted epididymus and coiled deferent ducts. Elongated and calcified claspers	Non-pregnant females with large ovaries and vitellogenic mature oocytes; developed but empty uteri
	Stage 4	—	Pregnant females with small ovaries and eggs in uterus, but non-differentiated embryos
	Stage 5	—	Pregnant females with small ovaries and differentiated embryos in the uteri
	Stage 6		Non-pregnant females with thin and distended uteri

Fertility was estimated by counting the number of embryos, and fecundity as the number of ovarian follicles developed before ovulation. The number of embryos per litter was plotted against female L_T. Sex ratio in embryos was also estimated and compared against a 1:1 proportion using the χ^2-test (Zar, 1984).

All the procedures in this investigation were designed and conducted according to the ethical standards of this journal (Editorial, 2006) and law of Argentina.

RESULTS

Data from 656 *Z. brevirostris* (320 males and 336 females) were analysed. Males ranged from 196 to 647 mm L_T and females ranged from 200 to 674 mm L_T, with the majority of individuals ranging from 450 to 620 mm L_T and 500 to 640 mm L_T, respectively (Fig. 2). The L_T and M_T relationship showed significant differences between sexes ($F = 275$, d.f. $= 1,632$, $P < 0.001$) and was described by the equation $M_T = 3\text{E-}05L_T^{2.730}$ ($r^2 = 0.971$, $P < 0.001$) for males and $M_T = 9\text{E-}06L_T^{2.963}$ ($r^2 = 0.971$, $P < 0.001$) for females. Sex comparison of the M_T and M_L relationship also showed significant differences ($F = 248$, d.f. $= 1,631$, $P < 0.001$).
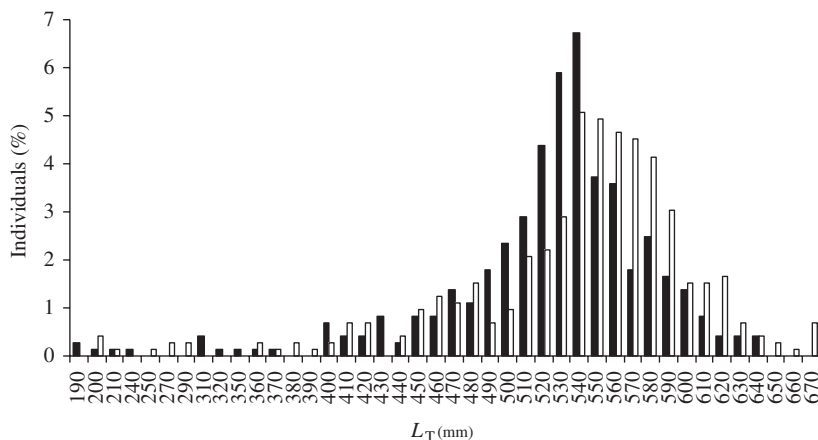
FIG. 2. Total length (L_T) frequency distribution of male (■; $n = 338$) and female (□; $n = 336$) *Zapteryx brevirostris* analysed between 2003 and 2007.

Females weighed more than males and the livers were heavier for females than males at any given L_T.

The smallest mature male measured 462 mm L_T and the largest juvenile 572 mm L_T (Fig. 3). Clasper length increased sharply between 470 and 550 mm L_T and clasper length of different mature stages overlapped between 500 and 600 mm L_T [Fig. 3(a), (b)]. A wide range of I_G values by size class was observed in mature
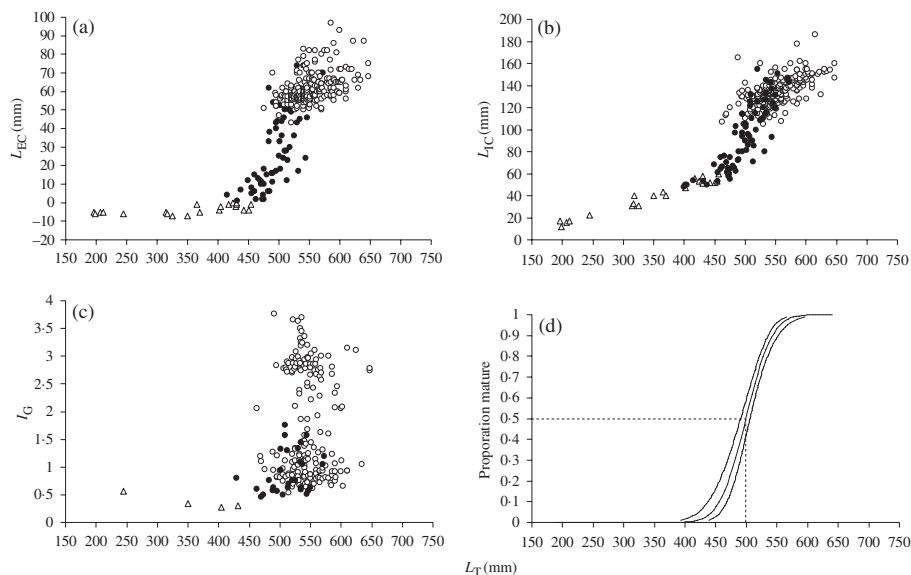

FIG. 3. Relationships between total length (L_T) and (a) external clasper length (L_{EC}), (b) internal clasper length (L_{IC}), (c) gonado-somatic index (I_G) (△, stage 1; ●, stage 2; ○, stage 3) and (d) L_T at maturity ogive with 95% CI of male *Zapteryx brevirostris*.
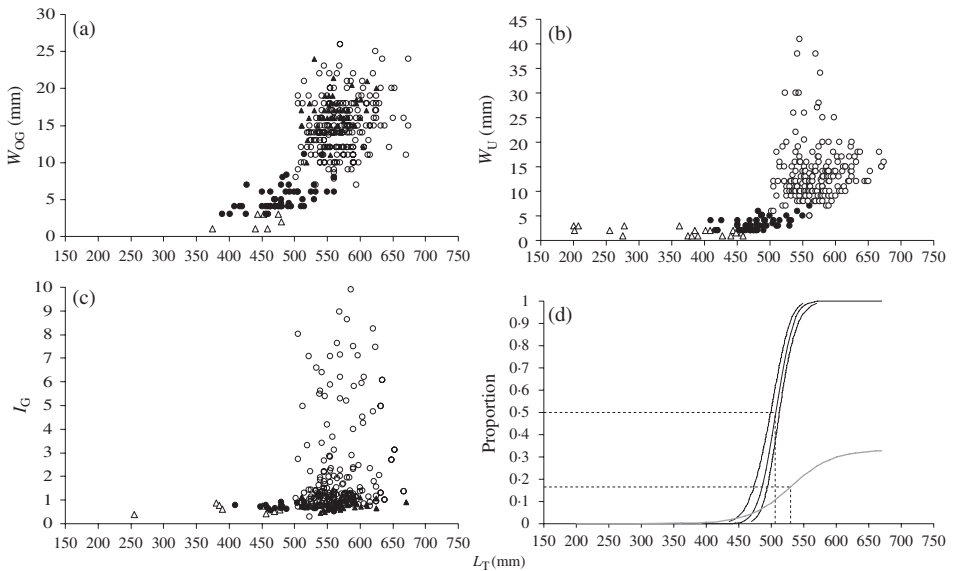
FIG. 4. Relationships between total length (L_T) and (a) oviducal gland width (W_{OG}), (b) uterus width (W_U), (c) gonado-somatic index (I_G) (△, stage 1; ●, stage 2; ○, stage 3; ▲, stage 4) and (d) L_T at maturity ogive (——) with 95% CI and maternity ogives (——) of female *Zapteryx brevirostris*.

individuals [Fig. 3(c)]. Mean L_{T50} was estimated to be 499 mm, representing 77% of the maximum L_T recorded for males [Fig. 3(d)].

On the basis of the abrupt change in W_{OG}, W_U and I_G with L_T, the range between the smallest mature female and the largest juvenile was from 502 to 560 mm L_T (Fig. 4). Mature females showed a considerable W_U variability by L_T class, differentiating a group of individuals with $W_U > 25$ mm [Fig. 4(b)]. These latter females, which had irrigated and flaccid uteri and ovaries with small ovarian follicles, appeared to be females in recent post-partum condition (stage 6). The L_{T50} was estimated to be 506 mm [Fig. 4(d)], which corresponds to 75% of the maximum L_T recorded for females. Females >560 mm were sexually mature and pregnant females (stages 4 and 5) ranged from 510 to 625 mm L_T. The L_{T50} of these fish was estimated to be 530 mm [Fig. 4(d)]. No significant differences were observed in the L_{T50} between sexes ($t = 0.774$, d.f. $= 1$, $P > 0.05$). The overall L_{T50} for both males and females was estimated to be 502 mm L_T.

Statistical analyses showed significant differences in the mean I_G values of males throughout the year ($F = 291$, d.f. $= 7,169$, $P < 0.001$), with minimum values during autumn and winter (May to August), medium during spring (November to December) and maximum during summer (February to March) [Fig. 5(a)]. The mean values of the I_H also varied significantly throughout the year; however, great monthly variation was observed [Fig. 5(b)].

Males had two functional and synchronic testes of the compound type (Pratt, 1988). Three seasonal patterns were related to the development of spermatocysts and the presence of spermatozoa in male genital ducts (Fig. 6): spring, testes with the majority of spermatocysts immature (cytes I and II) and empty genital ducts; summer and autumn, a higher proportion of spermatocysts after the spermatogenesis stage
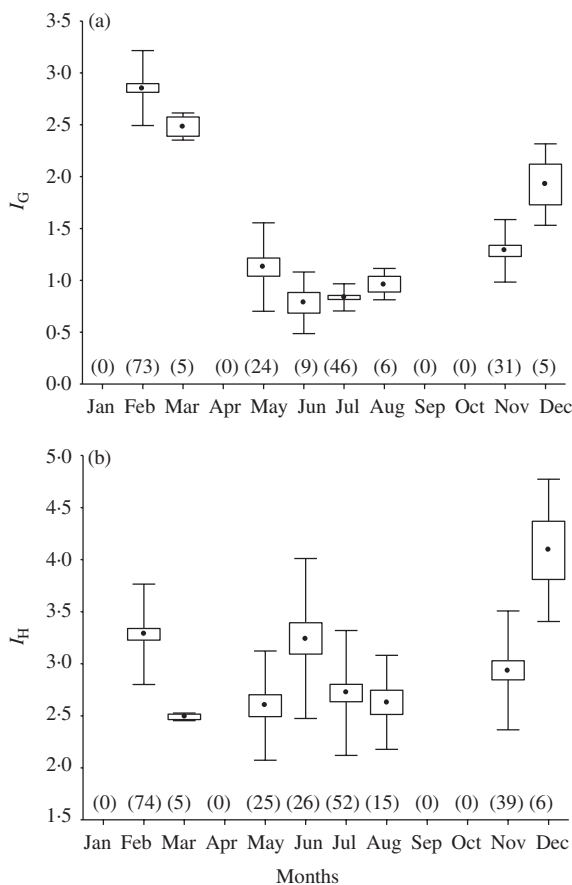
FIG. 5. Monthly variation of (a) gonado-somatic index (I_G) and (b) hepato-somatic index (I_H) of mature male *Zapteryx brevirostris*. For each month, the median (•), S.E. (□), S.D. (⊥) and the number of analysed samples in parenthesis are given.

with spermatozoa at different stages of aggregation and the lumen of ducts with small quantities of desegregated spermatozoa; and winter, testes with all spermatocysts immature, but genital ducts filled with spermatozoa in various stages of development. During the winter months males had testicular tubules, ventral to the testes lobes, full of disaggregated spermatozoa (Fig. 7).

On the basis of monthly variation in I_G and D_{LOF}, two groups of adult non-pregnant females could be distinguished [Fig. 8(a), (b)]. The intercepts of I_G and D_{LOF} were significantly higher for each month in one group than in the other. Females with the highest D_{LOF} were observed in August and females with eggs *in utero* were observed from August to November. Two females with presumptive pre-ovulatory follicles and eggs *in utero* were still observed in November [Fig. 8(a), (b)]. Females with uterine eggs contained within a brown-coloured proteinaceous membrane were collected from winter to spring months. The smallest embryos were observed in November and the largest ones in June. Females in presumptive post-partum condition were observed in July and August [Fig. 8(a), (b)]. Because
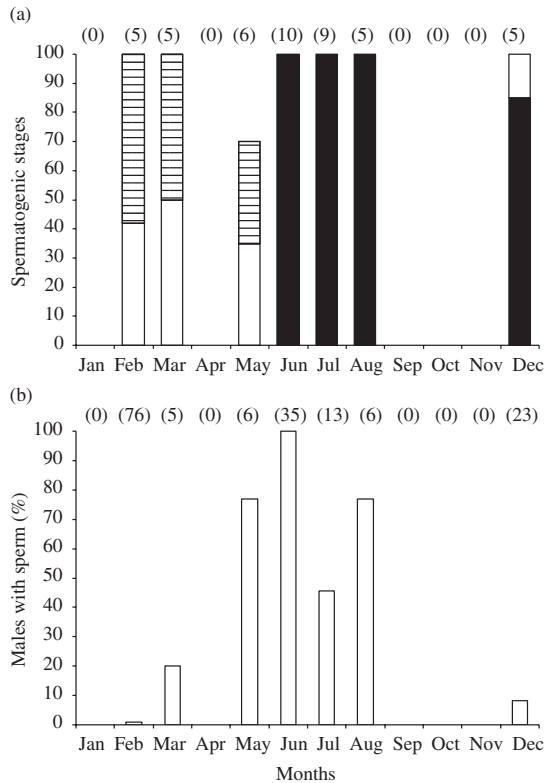
FIG. 6. Monthly variation in (a) spermatogenic stages [maturing (□), immature (■) and mature (▤)] and (b) per cent of mature male *Zapteryx brevirotris* with spermatozoa in deferent ducts. Numbers in parentheses are number of samples examined.

of the presence of females with maximum embryo size and females in post-partum condition, birth was assumed to occur during the winter months. No significant increment of follicle diameters was observed in pregnant females throughout the year [Fig. 8(b)].
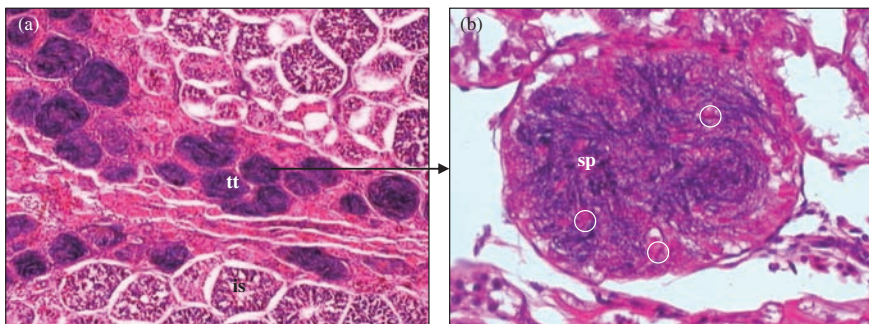

FIG. 7. Transverse section of the testis of a mature male *Zapteryx brevirostris* stained with haematoxylin and eosin, showing (a) immature spermatocyst (is) and testis tubules (tt) (magnification ×10) and (b) filled with spermatozoa (sp) and Sertoli cells (○) (magnification ×20).
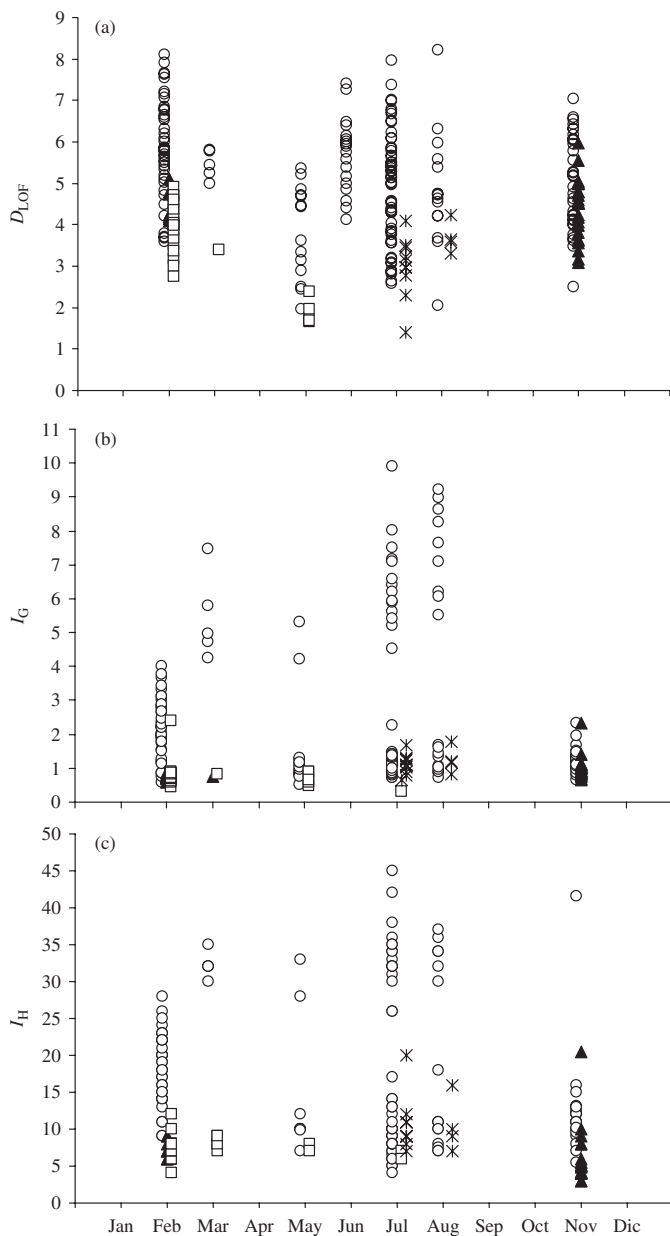
FIG. 8. Monthly variation of (a) the largest ovarian diameter (D_{LOF}) and (b) gonado-somatic index (I_G) and (c) hepato-somatic index (I_H) of mature female *Zapteryx brevirostris* (○, stage 3; ▲, stage 4; □, stage 5; ✳, stage 6).

The I_H increased in non-pregnant females with small ovarian follicles and decreased in those with large ovarian follicles [Fig. 8(c)]. After ovulation, I_H values were lower than those of non-pregnant adult females [Fig. 8(c)].

The number of embryos per month was insufficient for fitting a growth model. Fertility ranged from three to six embryos (mean ± S.D. = 3·8 ± 0·7), yet two embryos

per uterus were generally observed, and no significant correlation was observed between L_T of fertile females ($r^2 = 0.257$) and embryo size ($r^2 = 0.066$). The sex ratio of litters was not significantly different from 1:1 ($\chi^2 = 30.745$, d.f. $= 1$, $P < 0.001$).

DISCUSSION

Viviparous sharks (Cortés, 2000) and rhinobatids (Abdel-Aziz *et al.*, 1993; Capapé & Zaouali, 1994; Seck *et al.*, 2004; Enajjar *et al.*, 2008), unlike oviparous elasmobranchs (Ebert *et al.*, 2008), usually exhibit sexual dimorphism with females maturing and growing to a larger size than males. Species with this type of dimorphism show a positive correlation between litter size and female size. In *Z. brevirostris*, the lack of correlation between number and size of embryos and L_T of females could explain the similarity between sexes as far as maximum size and sexual maturity are concerned.

Both maximum L_T and L_{T50} estimated in this study proved to be higher than those estimated for Ubatuba Bay (latitudes $23°-24°$ S) (Ponz Louro, 1995; Abilhoa *et al.*, 2007), thus supporting the hypothesis of latitudinal change of some life-history variables. In addition, reproductive seasonality also differs geographically. In Brazil, although the cycle is not completely delineated, mature females are not reproductively synchronous (Batista, 1991; Ponz Louro, 1995; Abilhoa *et al.*, 2007). South of latitude $34°$ S the birth of embryos occurs during the winter months. Differences in the oceanographic conditions between these regions could be a selective pressure that results in phenotypic variation, but no geographical barriers are present that suggest population isolation. Several elasmobranchs occur in southern Brazilian and northern Uruguayan waters, but not off Argentina (Menni & Stehmann, 2000; Menni *et al.*, 2009), perhaps because the Rio de La Plata river discharge, one of the most important estuarine environments in the continent (Guerrero *et al.*, 1997; Mianzan *et al.*, 2001; Piola *et al.*, 2005, 2008), may act as a geographical barrier as suggested by Briggs (1974). *Zapteryx brevirostris*, however, has a continuous distribution from Brazil to Argentina, indicating that the environmental conditions do not influence all elasmobranchs in the same manner.

In the south-western Atlantic Ocean, a latitudinal increase in the maximum L_T and L_{T50} was also observed in the angular angel shark *Squatina guggenheim* Marini (Colonello *et al.*, 2007; Awruch *et al.*, 2008). This increase was related to the ability of larger individuals at higher latitudes to store energy for the low resource availability season (Colonello *et al.*, 2007). Regional differences of the reproductive events observed in *Z. brevirostris* could represent modifications of tactics (Wootton, 1984), within the same reproductive strategy, as a result of interactions between environment, behaviour, physiology and genetics.

Four different reproductive stages were distinguished in mature *Z. brevirostris* females: (1) non-pregnant with small ovarian follicles, (2) non-pregnant with large ovarian follicles, (3) pregnant with undifferentiated embryos and (4) pregnant with macroscopically differentiated embryos. The first two stages of ovarian development take 1 year each, and 2 years pass before ovulation occurs.

As evidenced in other guitarfishes (Lessa *et al.*, 1986; Rossouw, 1987; Abdel-Aziz *et al.*, 1993; Wenbin & Shuyuan, 1993; Capapé & Zaouali, 1994; Seck *et al.*,

2004; Marshall *et al*., 2007; Blanco Parra *et al*., 2009; Kume *et al*., 2009) and angel sharks (Capapé *et al*., 1990, 2002; Colonello *et al*., 2007), embryonic diapause was observed in *Z. brevirostris*. The duration of diapause varies between species and is regarded as a strategy to enable young to be born when environmental conditions are optimal (Marshall *et al*., 2007). It seems plausible that diapause may also be related to onshore migrations because *e.g.* females of *R. horkelii* take part in onshore–offshore migrations related to their reproductive cycle. During the autumn and winter months, females of *R. horkelii* in arrested zygote stage (diapause) are observed at *c*. 40 m, and during the spring–summer months they migrate to coastal waters (<10 m) when rapid embryonic growth occurs (Lessa *et al*., 1986). Vitellogenesis continues during the diapause stage in guitarfishes, which implies a higher energy demand.

The breeding season of *Z. brevirostris* in the study area takes place during the winter months. This behaviour contrasts with other regional viviparous elasmobranchs, like the narrownose smoothhound *Mustelus schmitti* Springer (Menni *et al*., 1986; Cortés, 2007) and *S. guggenheim* (Colonello *et al*., 2007). These sharks breed in coastal waters during late spring (December) when environmental conditions appear to be optimal and during a period of abundant prey availability. Breeding during the winter months probably endows *Z. brevirostris* with some kind of adaptive advantage, through reduction of predation risk and interspecific competition. These factors could increase growth rates and reduce the time they are vulnerable (Heupel *et al*., 2007).

Kyne & Bennett (2002) proposed that rhinobatid species can be divided into two groups according to their mode of reproduction. In one group, vitellogenesis does not proceed in parallel with gestation, and birth seasonality differs from ovulation and mating. In the other group, vitellogenesis proceeds in parallel with gestation, and birth and subsequent copulation are not seasonally distinct. The reproductive strategy of the second group implies a 1 year female reproductive cycle, a high ovary developmental rate and a rapid embryonic growth. These rhinobatids produce within 6 months a batch of 30–40 mm diameter ovarian follicles, and the embryonic development, after the diapause period, appears to last from 3 to 5 months (Lessa *et al*., 1986; Wenbin & Shuyuan, 1993; Capapé & Zaouali, 1994; Villavicencio-Garayzar, 1995; Kyne & Bennett, 2002; Seck *et al*., 2004; Ismen *et al*., 2007; Márquez-Farías, 2007; Marshall *et al*., 2007; Blanco Parra *et al*., 2009). In sharks, the reproductive cycle duration is related to the largest size of ovarian follicles (Walker, 2005). This hypothesis is supported by regional examples. Species like *M. schmitti* (Menni *et al*., 1986; Cortés, 2007), with follicles <30 mm, show a 1 year reproductive cycle whereas species like the broadnose sevengill shark *Notorynchus cepedianus* (Péron) (Ebert, 1996; Lucifora *et al*., 2005), with follicles >30 mm diameter, have a 2 year reproductive cycle and even species like the school shark *Galeorhinus galeus* (L.) (Peres & Vooren, 1991; Lucifora *et al*., 2004; Walker, 2005) and *S. guggenheim* (Colonello *et al*., 2007) show a 3 year reproductive cycle. On the basis of the reproductive classification of rhinobatids (Kyne & Bennett, 2002), *Z. brevirostris* might belong to the group where vitellogenesis does not proceed in parallel with gestation, which implies that birth and hence mating are not simultaneous processes. This grouping, however, does not consider multi-annual reproductive cycles. Therefore, this classification should be reconsidered. *Zapteryx brevirostris* shares the same reproductive pattern of a 3 year ovary development (2 years of mature non-pregnant and 1 year of pregnant) and a 1 year gestation period with two regional sharks: *G. galeus* (Peres & Vooren, 1991;

Lucifora *et al*., 2004; Walker, 2005) and *S. guggenheim* (Colonello *et al*., 2007). These species are neither closely related nor are they similar in their reproductive mode, which indicates that the pattern must have evolved several times independently and is not dependent on reproductive mode (Colonello *et al*., 2007).

Mature females have high I_H values during the second year of the ovarian cycle, when it begins to decrease at the same time as ovarian follicle diameters increase, and pregnant females (stages 4 and 5) show the lowest I_H values. Liver energy could be used as a source of energy and lipids for follicles that develop during the second year. The small livers during gestation cannot, however, be explained by embryonic nourishment because of the lecithotrophic mode.

Testes of elasmobranchs have been classified into three types in accordance with spatial patterns in the formation of spermatocysts during developmental stages (Pratt, 1988). Testes of *Z. brevirostris*, as observed for other rhinobatids (Wenbin & Shuyuan, 1993; Kyne & Bennett, 2002; Kume *et al*., 2009; Yamaguchi & Kume, 2009), belong to the compound type where the lobes develop radially and spermatocysts migrate along the diameter.

Studies on male elasmobranchs do not support the assumption that relative gonad size (I_G) and reproductive readiness are positively correlated (Parsons & Grier, 1992; Maruska *et al*., 1996; Sulikowski *et al*., 2004, 2005; Kume *et al*., 2009; Yamaguchi & Kume, 2009). In *Z. brevirostris*, three reproductive patterns were identified. During the spring months (November to December), males have medium-size testes with immature spermatocysts and empty deferent ducts. During summer to autumn (February to May), males exhibit the highest I_G and have testes with mature and maturing spermatocysts although no spermatozoa are present in the reproductive ducts. During winter, I_G is lowest and spermatocysts are immature, but the lumen of the genital ducts is full of spermatozoa at different stages of aggregation. Ventral to the lobes, the testicular tubules are filled with disaggregated spermatozoa. The co-occurrence of immature spermatocysts (cytes I and II) and disaggregated spermatozoa in the testicular tubules suggest intra-testicular spermatozoa storage. These sort of tubules have not been described in other Chondrichthyes. The presence of spermatozoa in testicular tubules may relate to storage of batches of spermatozoa because species with high copulation frequencies need mechanisms to prevent liberation of the entire spermatozoa production at one time. The observed spermatozoa production, and their storage in *Z. brevirostris*, suggests a strategy that would allow males to gain access to several females over a short period of time.

Synchronicity of both ovulating females and males with spermatozoa in genital ducts indicate that ovulation and hence fertilization occurs during the winter months. No injuries were observed in mature females that suggest male biting or holding.

The L_T at maternity was larger than L_T at maturity. Although all females >560 mm L_T were mature, about one-third of the population was in maternal condition and hence contributing to annual recruitment. For chondrichthyan species with a 1 year continuous reproductive cycle, calculation of population size is possible using maturity or maternity ogives because all mature females contribute to annual recruitment each year (Walker, 2005). For species with a reproductive cycle of >1 year, however, such as *Z. brevirostris*, it is necessary to estimate the maternity ogive independently of the maturity ogive.

A low reproductive frequency has direct implications for annual fecundity. A 3 year reproductive cycle results in a mean annual fecundity of one to two embryos.

These fecundity values are smaller than those of other regional rhinobatids (Lessa *et al*., 1986) or even lecithotrophic chondrichthyans with a 3 year reproductive cycle (Peres & Vooren, 1991; Lucifora *et al*., 2004; Colonello *et al*., 2007). The reproductive efficiency of *Z. brevirostris* appears to be lower than that of matrotrophic or even other lecithotrophic elasmobranchs.

South of latitude 34° S, *Z. brevirostris* is caught by a multispecies fishery and entirely discarded because of lack of commercial value. It is reasonable to think that the impact of this fishery on *Z. brevirostris* is low, but post-release survival is unknown. Declines in abundance in the region (Massa *et al*., 2004), together with a low reproductive capacity, mean that the *Z. brevirostris* population should be closely monitored. Survivorship studies on discarded batoids are needed to evaluate human impact on these species because despite their low economic value, they may be ecologically significant, possibly keystone species, and hence fishery impacts may be disproportionately high.

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