

Reproductive biology of the skate, *Rioraja agassizii* (Müller and Henle, 1841), off Puerto Quequén, Argentina

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Summary

A total of 230 specimens of the skate, *Rioraja agassizii*, was collected from the commercial bottom trawl fishery of Puerto Quequén, Argentina. During the warmer seasons greater proportions of females were sampled. The largest male and female sampled were 629 and 698 mm total length (TL), respectively. Sexual dimorphism was detected in the relationship between weight (W)–TL and disc width (DW)–TL, in that females were heavier and wider than males. The smallest mature male and female were 485 and 530 mm TL, respectively. TL at 50% maturity ($TL_{50\%}$) was estimated at 504 and 570 mm for males and females, respectively. Females exhibited heavier livers than males throughout their lifetimes. Seasonal variations in the gonadosomatic (GSI) and hepatosomatic (HSI) indices, and in the width of the left oviducal gland (OG), along with the greater number of pregnant females sampled during spring, suggested that the peak of the egg laying season could be during this season. The egg cases presented a mean length of 69.01 ± 5.53 mm, mean width of 43.40 ± 1.86 mm and mean weight of 17.00 ± 4.59 g. According to these results specimens from Puerto Quequén and southern Brazil appeared to differ in size, the peak of the egg-laying season and in egg dimensions.

Introduction

Elasmobranchs have become an increasing component in fisheries worldwide and a valuable resource for most Argentine trawl fisheries (Tamini et al., 2006). Until 1990 in Argentina, batoid species were considered as bycatch and discarded at sea due to their low economic value (Chiaramonte, 1998; Cousseau et al., 2000). During the 1990s this situation was reversed, with commercial landings increasing from 300 tonnes year⁻¹ in 1991 to 14 856 tonnes year⁻¹ in 1998 (Cousseau et al., 2000). From 2001 to 2004 commercial landings oscillated between 14 700 tonnes year⁻¹ and 18 100 tonnes year⁻¹, reaching 22 000 tonnes year⁻¹ in 2005 (Dirección Nacional de Pesca).

Studies on batoids in Argentina have focused on taxonomy and distribution of the species (Menni and Stehmann, 2000). A quantitative approach to the study of reproduction is a critical aspect required for fisheries management (Walker, 2005). In Argentine fisheries, the tool established by the national fisheries authorities to manage the resources is the Total Allowable Catch (TAC). For batoids, it is conducted without discrimination of species. This situation and the lack of biology information of skate species make it difficult to develop adequate management tools for these species.

Rioraja agassizii (Müller and Henle, 1841) is an endemic skate in the southwestern Atlantic, and the only known species

of the genus *Rioraja*. It is distributed from Rio de Janeiro, Brazil, to northern Patagonia, Argentina (Figueiredo, 1977; Menni and López, 1984). Its distribution comprises the South Brazilian and Bonaerensean districts (Menni, 1973; Menni and Stehmann, 2000). It is a coastal species, found from 10 to 90 m depth and at water surface temperatures from 6 to 10.5°C (Menni and López, 1984).

In Buenos Aires province, during the 2000–2006 period, the coastal fishery operating from Puerto Quequén contributes, on average, 3.9% of the total batoids landed in Argentina (Dirección Nacional de Pesca). In this multispecies bottom trawl coastal fishery, *R. agassizii* is part of the landed commercial species, with the undersized specimens considered as bycatch and discarded (Tamini et al., 2006). To date no biological information is available for this species in Argentina. The aim of this work is to contribute to the knowledge of the reproductive biology of *R. agassizii* in the area of Puerto Quequén.

Materials and methods

Sampling

A total of 230 specimens of *R. agassizii* comprising 93 males and 137 females was collected from commercial landings of the bottom trawlers operating at Puerto Quequén (58°50'W – 38°37'S), Buenos Aires province, Argentina. Samples were taken during autumn (2000 and 2001), winter (2000, 2001 and 2005), spring (2001 and 2004) and summer (2001 and 2005).

Morphometrics and sex ratio

All sampled specimens were intact, without broken tails. Each specimen was sexed; total length (TL) and disc width (DW) were recorded to the nearest mm. Length–frequency distribution was assessed by pooling the data for 20 mm size classes. Total fresh weight (W) was registered with a precision of 50 g and the liver weighted (W_L) to the nearest 0.1 g. The reproductive tracts were removed and frozen until processed in the laboratory. The relationships W –TL and DW–TL were estimated by regressions for each sex and compared with a Student's t -test for slopes and elevations (Zar, 1996). Non-parametric test was used to detect differences in sex ratio in the whole sample as well as seasonally (Zar, 1996).

Length at maturity and reproduction

In order to characterize the maturity in both sexes, measurements of the reproductive tracts were made with callipers to the nearest 0.1 mm, and weights recorded with a precision of

0.1 g. In both sexes the size of the smallest mature specimen was recorded. Length at 50% of maturity ($TL_{50\%}$) was estimated following Roa et al. (1999).

Males were categorized as: immature I (skeleton of the claspers were non-calcified and testes undeveloped); immature II (claspers partially calcified and testes developing); and mature [claspers fully calcified and the base of them could be rotated directing it anteriorly, testes completely developed and sperm ducts coiled (Clark and von Schmidt, 1965)]. Clasper lengths were measured from the end of the cloacae to the tip of the claspers and plotted against TL. Testes were weighed (W_G) and differences between right and left testes tested by Student's t -test for dependent samples (Zar, 1996).

Females were categorized as: immature I (thin tubular oviducts and undeveloped ovaries); immature II (thin tubular oviducts and developing ovaries with no yolk follicles); and mature (enlarged oviducts and ovaries with yolk follicles, with or without egg cases in oviducts) (Stehmann, 2002; San Martín et al., 2005). Each egg case was weighed. Length without horns and maximum width were registered following Hubbs and Ishiyama (1968).

Width of each oviducal gland (OG), weight of the ovaries (W_G) and diameter of the largest follicles were recorded. Differences in width between right and left OG and in weight between right and left ovaries were tested by Student's t -test for dependent samples (Zar, 1996). Width of the left OG for mature females was tested for seasonal variations by Kruskal–Wallis (H) non-parametric ANOVA. Where differences were significant, multiple comparisons (Q) were performed (Zar, 1996).

The gonado-somatic index (GSI) was calculated ($GSI = \frac{W_G}{W} \times 100$). In mature specimens seasonal differences in the GSI values were tested with a Kruskal–Wallis (H) non-parametric ANOVA. Where differences were significant, multiple comparisons (Q) were performed (Zar, 1996).

The W_L –TL relationship were estimated by regressions for each sex and compared with a Student's t -test for slopes and elevations (Zar, 1996). The hepatosomatic index (HSI) was calculated ($HSI = \frac{W_H}{W} \times 100$). In order to detect differences in the HSI between immature and mature specimens in both sexes, a Student's t -test was performed (Zar, 1996). Seasonal differences for immature and mature individuals were tested with a Kruskal–Wallis (H) non-parametric ANOVA. Where differences were significant, multiple comparisons (Q) were performed (Zar, 1996). Since not enough data were available for mature specimens during autumn, this season was excluded for the previous seasonal analysis (OG, HSI and GSI).

Results

Sizes and sex ratio

Size ranges of the specimens sampled were 258–629 mm TL and 277–698 mm TL for males and females, respectively (Fig. 1). Weight ranges were 60–1350 g for males and 68–1950 g for females. The W –TL relationship for males and females is shown in Fig. 2. Elevations of the log-transformed relationship between W –TL were significantly different between sexes (d.f. = 227; $t = 17.77$; $P < 0.05$). This result was due to differences in elevations in mature specimens (d.f. = 113; $t = 7.32$; $P < 0.001$), since no differences were found between immature specimens ($P > 0.05$).

The relation DW–TL was significantly different between sexes (d.f. = 202; $t = 2.10$; $P < 0.05$), with females larger, at a given TL, than males (Fig. 3). This result was due to

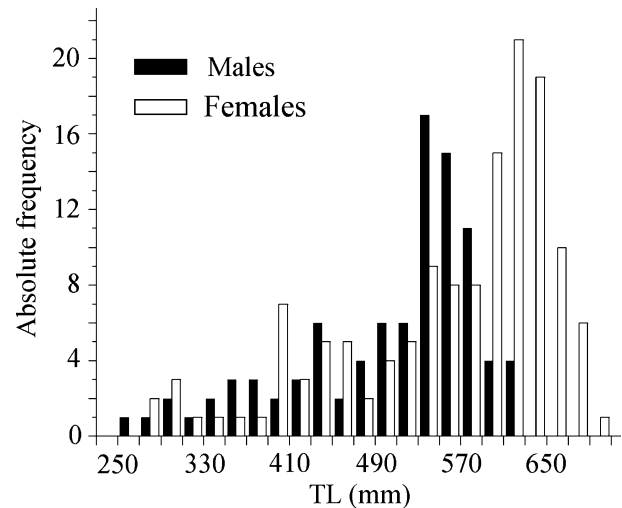


Fig. 1. Length–frequency distribution of *R. agassizii* using all study data, 2001 to 2005. Data are presented for 20 mm size classes; females $n = 137$; males $n = 93$

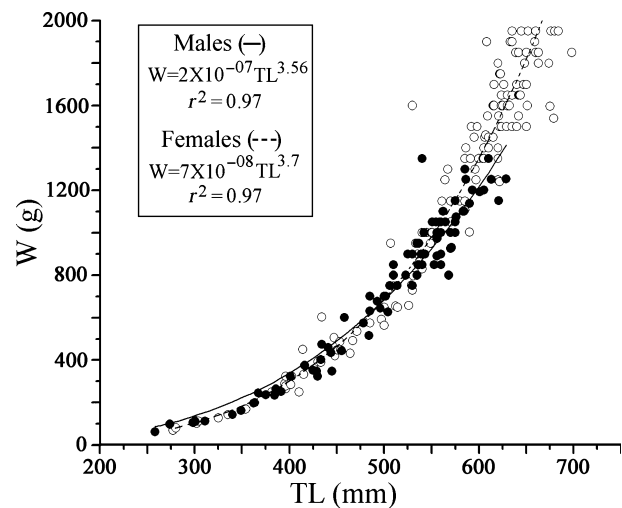


Fig. 2. Relation between weight (W) and total length (TL) in *R. agassizii*. Females (○) $n = 137$; males (●) $n = 93$

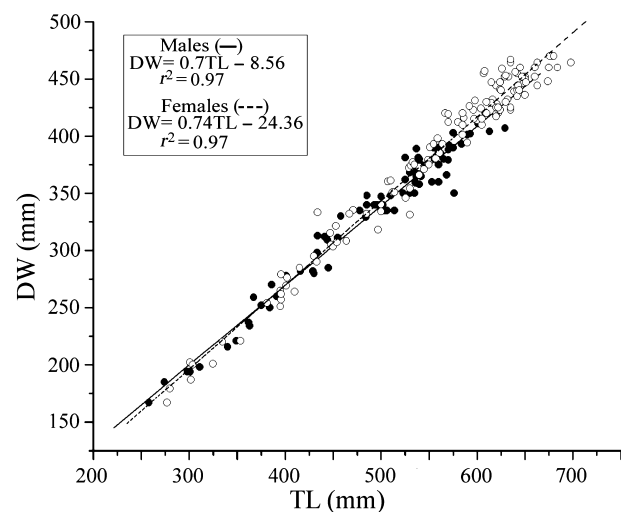


Fig. 3. Relation between disc width (DW) and total length (TL) in *R. agassizii* off the Province of Buenos Aires coast. Males (●) $n = 80$; females (○) $n = 126$

differences in elevations in mature specimens (d.f. = 94; $t = 3.19$; $P < 0.0001$), because no differences were found between immature specimens ($P > 0.05$).

The proportion of females was significantly different from that expected for the whole sample (1.00 : 1.47; $\chi^2 = 8.42$; $P < 0.05$). Greater proportions of females were observed during spring and summer (1.00 : 1.60; $\chi^2 = 4.65$; $P < 0.05$ and 1.00 : 2.33; $\chi^2 = 11.20$; $P < 0.05$, respectively).

Length at maturity and reproduction

All males < 485 mm TL were immature, having uncalcified clasper skeletons (stages I and II). The smallest mature male was 485 mm TL, representing 70.3% TL of the largest male sampled. Transition from immaturity to maturity was between 485–590 mm TL. All males > 590 mm TL (85.5% TL of the largest male sampled) were mature with claspers fully calcified (Fig. 4).

All females < 530 mm TL were immature (stages I and II) presenting undeveloped ovaries and no yolk follicles. The smallest mature female sampled was 530 mm TL (76.0% TL of the largest female sampled) and was carrying egg cases. All females > 629 mm TL were mature (90.1% TL of the largest female sampled).

The estimated TL_{50%} varied between sexes. For males, the estimated TL_{50%} was 504 mm, representing 80.1% TL of the largest male sampled (Fig. 5). For females, TL_{50%} was 570 mm, representing 81.7% TL of the largest female sampled (Fig. 5).

In males, claspers length increased abruptly between 480 and 500 mm TL (Fig. 4). No significant differences between right and left testes weight were found ($P > 0.05$). In females, the maturity stage was characterized by the enlargement of the oviducal gland (> 39 mm) and completely developed oviducts and ovaries containing mature yolk oocytes (11.0–39.1 mm). No significant differences were found between weight of the right and left ovaries ($P > 0.05$). In mature females, the left OG was wider than the right OG (d.f. = 67; $t = 2.48$; $P < 0.05$). The left OG showed seasonal differences (d.f. = 2; $H = 12.92$; $P < 0.01$). Maximum OG diameters occurred during spring and winter ($P > 0.05$), showing the lowest value in summer (d.f. = 3; $Q_{Wi-Su} = 3.11$;

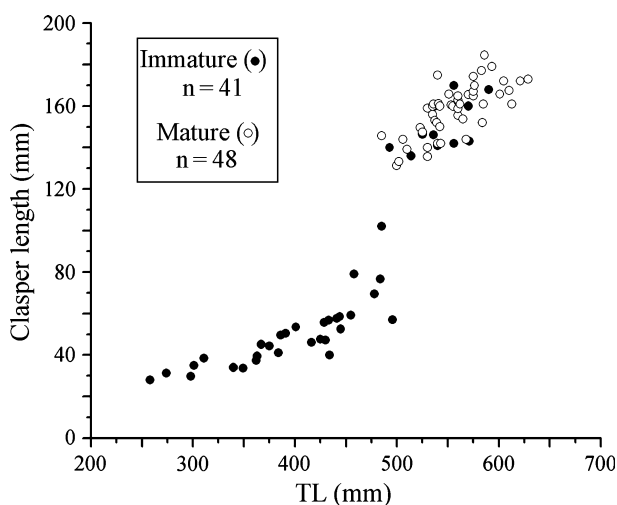


Fig. 4. Relation between clasper length and total length (TL) in mature and immature males of *R. agassizii*. Immature (●) n = 41; mature (○) n = 48

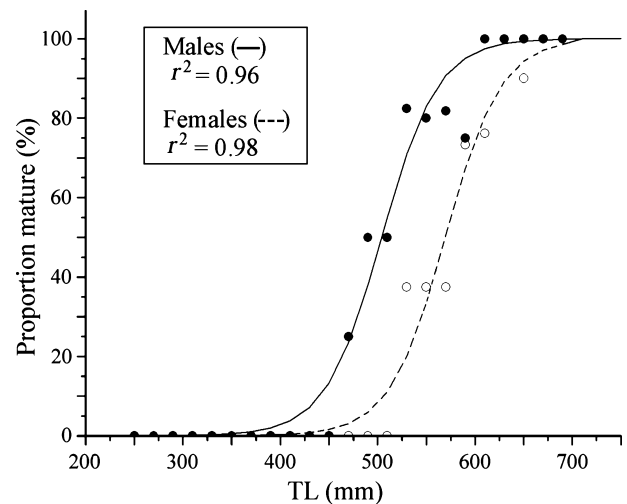


Fig. 5. Proportion of mature *R. agassizii* at given total length (TL) based on catches in 2001 and 2005. Lines represent length at maturity for males (●) and females (○)

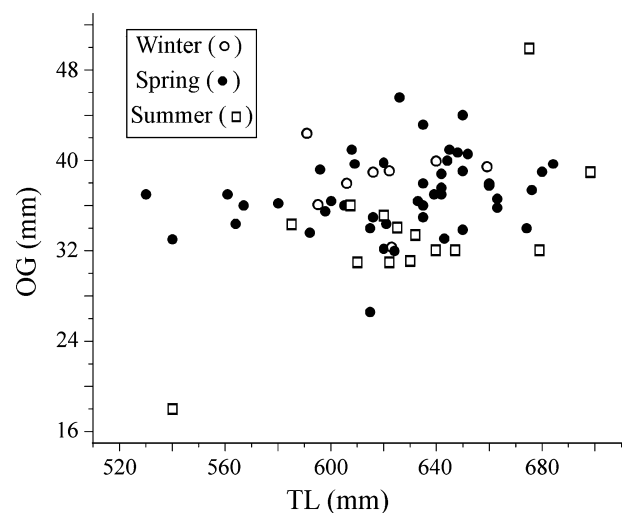


Fig. 6. Width of oviducal gland (OG) and total length (TL) of mature *R. agassizii* females in relation to seasons n = 8 (winter); n = 45 (spring); n = 14 (summer)

$Q_{Sp-Su} = 3.16$; $P < 0.05$). The relationship between left OG–TL is shown in Fig. 6.

Seasonal variations of immature and mature specimens sampled are shown in Table 1. The greatest proportion of females carrying egg cases was registered during spring: of over 47 mature females sampled, 25 (53%) carried egg cases

Table 1
Seasonal variation of mature and immature *R. agassizii* specimens, and mature females without and with egg capsules

Season	Immature		Mature		Mature females	
	Females	Males	Females	Males	Without capsules	With capsules
Autumn	20	19	0	2	0	0
Winter	7	7	8	11	7	1
Spring	6	5	47	28	22	25
Summer	34	13	15	8	13	2
Total	67	44	70	49	42	28

Table 2
Size and weight of egg capsules of *R. agassizii*. Comparison between specimens from Puerto Quequén and from south Brazil

	Present study				Oddone et al., 2006			
	Mean	Range	SD	Total	Mean	Range	SD	Total
Width (mm)	43.40	40–47.2	1.86	40	30.53	22–36	2.32	118
Length (mm)	69.01	57.1–79	5.53	31	47.34	41–56	3.09	119
Weight (g)	17.00	7.5–23	4.59	42	–	–	–	–

(Table 1). Among the 28 females carrying egg cases in all seasons, 22 (79%) carried egg cases that were completely developed.

Although the left OG was wider, no significant differences were found between egg cases in the left and right oviducts, in length, width or weight ($P > 0.05$). The egg cases presented a mean length of 69.01 ± 5.53 mm, mean width of 43.40 ± 1.86 mm and a mean weight of 17.00 ± 4.59 g (Table 2).

Although not enough data were available to develop a statistical comparison between seasons, the GSI values for mature males seemed to be constant throughout the year (Fig. 7). Seasonal variation in the GSI values was found for mature females (d.f. = 2; $H = 23.17$; $P < 0.0001$; Fig. 7). The GSI peak value was registered during spring (d.f. = 3; $Q_{Sp-Wi} = 2.61$; $Q_{Sp-Su} = 4.81$; $P < 0.05$) and the lowest during summer (d.f. = 3; $Q_{Su-Wi} = 2.48$; $P < 0.05$).

Females, at a given TL, displayed heavier livers than males throughout their lifetime (Fig. 8). The log-transformed relationship W_L -TL was significantly different between sexes (d.f. = 222; $t = 4.55$; $P < 0.001$). Differences were also observed when comparing immature (d.f. = 107; $t = 3.53$; $P < 0.001$) and mature specimens separately (d.f. = 111; $t = 2.94$; $P < 0.05$).

The hepatosomatic index varied according to the maturity condition of the specimens. HSI of the immature males was greater on average than that of mature males (d.f. = 91; $t = 2.14$; $P < 0.05$). In contrast, mature females showed

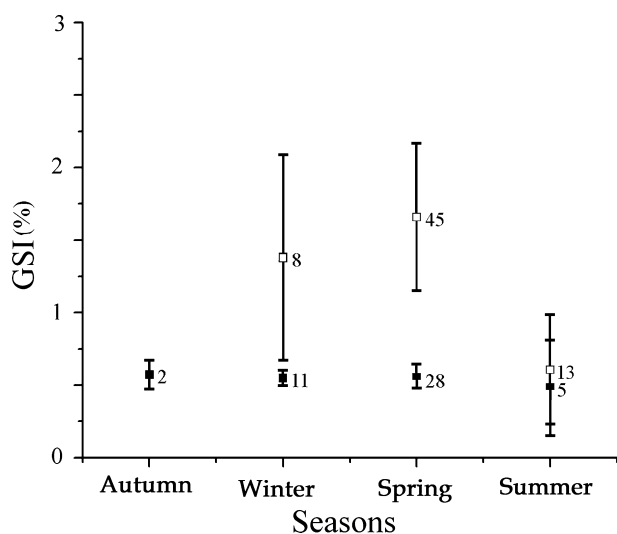


Fig. 7. Seasonal variation of the gonado-somatic index (GSI) in *R. agassizii* specimens caught off the Province of Buenos Aires coast. Mature males (■) and females (□). Squares correspond to means and bars to standard deviations. Numbers next to the data point indicate the respective n behind the data set

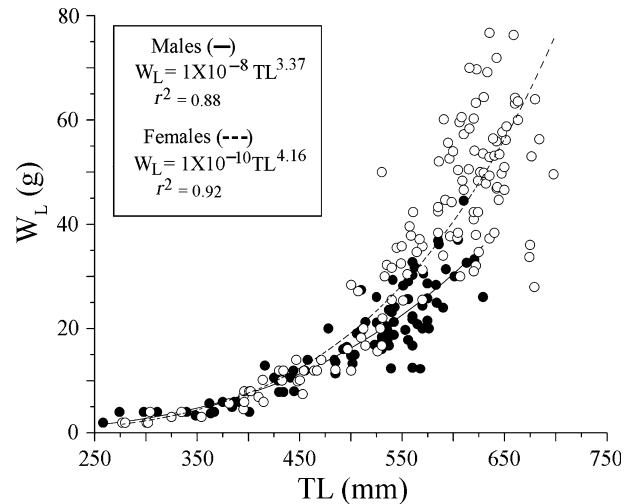


Fig. 8. Relation between liver weight (W_L) and total length (TL) in *R. agassizii*, Buenos Aires province coastal region. Males (●) $n = 93$ and females (○) $n = 133$

greater HSI values than immature ones (d.f. = 129; $t = 2.72$; $P < 0.01$). No seasonal variation of the HSI was detected in immature males and females ($P > 0.05$; Fig. 9a,b). Mature males and females exhibited seasonal variations in HSI values (d.f. = 2; $H = 16.54$; $P < 0.001$, for males; d.f. = 2; $H = 21.8$; $P < 0.001$ for females; Fig. 9a,b). In mature males, maximum HI values were detected during winter and summer ($P > 0.05$). The lowest value was registered in spring (d.f. = 3; $Q_{Sp-Su} = 2.42$; $Q_{Sp-Wi} = 3.76$; $P < 0.05$). In mature females the highest HSI value was detected during winter (d.f. = 3; $Q_{Wi-Sp} = 3.29$; $Q_{Wi-Su} = 4.67$; $P < 0.05$) and the lowest in summer (d.f. = 3; $Q_{Su-Sp} = 2.61$; $P < 0.05$).

Discussion

In our study larger specimens dominated the length frequency distribution of *R. agassizii*; this could be attributed to the fact that the bulk of the specimens came from commercial landings. Females attain larger sizes than males, as 26% of the females sampled were larger than the largest male.

A difference in W -TL relationship between sexes is commonly found in elasmobranchs. It was observed for *R. agassizii* that adult females were heavier at a given length than males. The same feature was also reported for other sympatric skate species (Braccini and Chiaramonte, 2002; Mabragaña et al., 2002; Mabragaña and Cousseau, 2004). Exceptions had been found in *Psammobatis bergi*, where females were heavier than males throughout their lifetime (San Martín et al., 2005) and in *Psammobatis rudis* where no intersexual differences were detected (Mabragaña and Cousseau, 2004). The DW-TL relationship also displayed sexual dimorphism in *R. agassizii*, with mature females broader than mature males at a given TL.

According to Springer (1967), unequal sex ratio could be a consequence of sexual segregation. As with *P. bergi* (San Martín et al., 2005), during the warmer seasons greater proportions of females were sampled. In this case, these seasonal changes in the proportions could reflect reproductive migratory movements and/or are a consequence of the selection process of larger specimens by fishermen for commercial purpose.

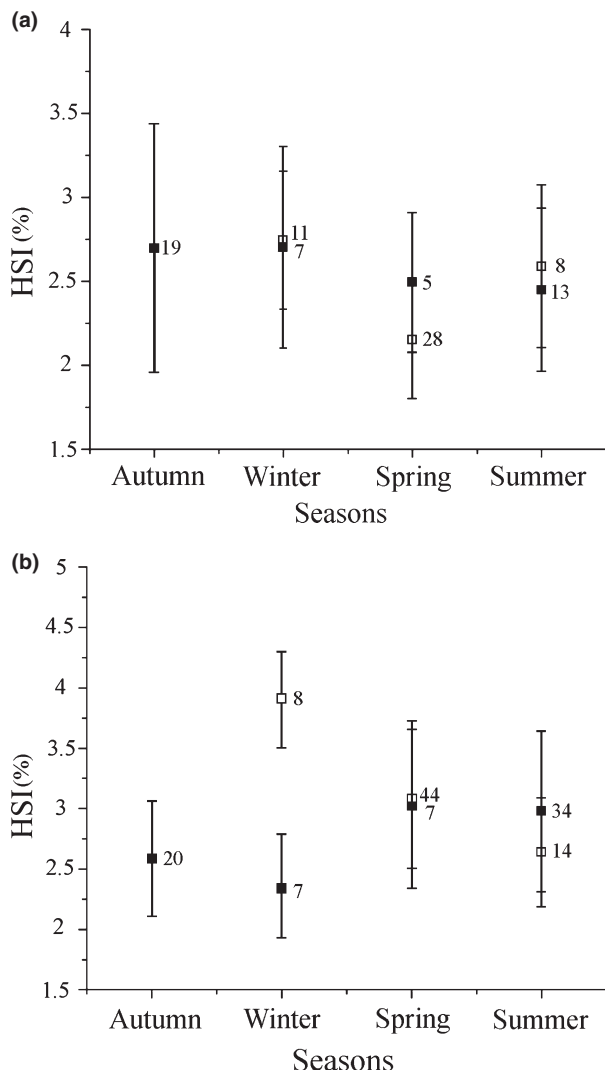


Fig. 9. Seasonal variation of hepatosomatic index (HSI). (a) = Immature (■) and mature (□) males. Squares correspond to means and bars to standard deviations; (b) Seasonal variation of hepatosomatic index. Immature (■) and mature (□) females. Squares correspond to means and bars to standard deviations. Numbers next to the data point indicate respective n behind the data set

Total length at first maturity for males and females in other common sympatric skate species were $75 \pm 5\%$ of the maximum TL observed (Braccini and Chiaramonte, 2002; San Martín et al., 2005). Females maturing at larger sizes than males are a common feature in elasmobranch species, the same as in *R. agassizii*. The estimated $TL_{50\%}$ differed between sexes with females maturing at larger size than males. For other skate species of the area, it was found that males mature at larger sizes than females (Braccini and Chiaramonte, 2002; San Martín et al., 2005). This indicates that sexual dimorphism in size at maturity is variable among sympatric species.

Most oviparous species of sharks and skates have a year round egg production, with seasonal periods when a greater proportion of adult females are laying eggs (Hamlett and Koob, 1999). Gonadosomatic indices are good indicators of the periodicity of reproduction (Jons and Miranda, 1997). In the present study, seasonal variations were found in the GSI and the OG. The GSI exhibited its maximum value during spring, the OG during winter–spring seasons. In addition, during spring about 50% of the mature females were repro-

ducing. According to our results, the egg laying period could occur in the area throughout the year, with the peak during spring. Off Brazil, this occurred from late spring to early winter, with a peak during summer (Menni and Stehmann, 2000). This lack of synchronization suggests geographical differences for the species along its latitudinal distribution.

In the area of Puerto Quequén, the egg cases exhibited a mean size of $c. 69 \times 43$ mm, whilst in southern Brazil the mean size was $c. 47 \times 30$ mm (Oddone et al., 2006). These differences in size could be attributed to: (i) the fact that southern reproducing females were larger than the Brazilian females (in Puerto Quequén, from 530–690mm TL, and in Brazil from $c. 400$ –600 mm TL; see Fig. 5 in Oddone et al., 2006); or (ii) different adaptations to their environments.

Sexual dimorphism in liver size is often reported in elasmobranchs (Rossouw, 1987). The liver is a key organ in female reproduction because it is involved in the production of vitellogenin, the yolk precursor (Koob and Callard, 1999). Females showed heavier livers than males at any given TL in *R. agassizii*, which points to their greater energetic requirements. Mature females also exhibited greater HSI values than immature females. This feature was given by the high value shown in winter, the season before the egg-laying peak. In males, the HSI showed higher values for immature than for mature males. This could be a consequence of the liver depletion in mature males exhibited in spring due to their reproduction investment.

In another study carried out in the area of Puerto Quequén, Perez Comesaña et al. (2006) found a reduction of 51 mm in the mean DW value of commercially landed *R. agassizii* between 2001 and 2005. This result was interpreted as a sign of over-exploitation which is a matter of concern. Finally, the statement of local differences in several reproductive parameters along the distribution of *R. agassizii* must be also taken into account for any management policy.

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