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Reproductive biology of the skates *Sympterygia acuta* Garman, 1877 and *S. bonapartii* Müller & Henle, 1841 (Chondrichthyes: Rajoidei) in south Brazil

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The present study analyzed the sexual development, sizes at maturity and morphometric relationships for both sexes of *Sympterygia acuta* and *S. bonapartii*, endemic of south-western Atlantic Ocean. The examined specimens were obtained through research cruises and commercial fishing trips, during 2011 and 2012, along the southern Brazilian coast, in latitudes ranging from 34°28'S to 31°29'S and at depths between 15 and 142 m. Significant differences ($p<0.05$) in *S. bonapartii* and in *S acuta* between sexes for the relationships total length (cm) - disc width (cm) and total length - total/eviscerated weight (g) respectively, demonstrated sexual dimorphism during the development in both species. The estimated size at maturity for males and females, respectively, were 46.1 and 44.7 cm, for *S. acuta*, and 58.4 and 59.9 cm, for *S. bonapartii*. A decrease in size at maturity for both sexes was observed in *S. acuta* and females of *S. bonapartii* of the southern coast of Brazil, respect to previous studies carried out over the last 30 years.

O presente estudo analisou o desenvolvimento sexual, os tamanhos de maturidade e as relações morfométricas para ambos os sexos das espécies *Sympterygia acuta* e *S. bonapartii* endêmica do sudoeste do Oceano Atlântico. Os indivíduos foram coletados em cruzeiros de investigação e viagens de pesca comercial durante 2011 e 2012 ao longo da costa do Sul do Brasil em latitudes que variam de 34°28'S a 31° 29'S, e em profundidades entre 15 e 142 m. Houve diferenças significativas entre sexos para *S. bonapartii* ($p <0,05$) e *S acuta* nas relações comprimento total (cm) - largura do disco (cm) e comprimento - peso total e comprimento total – peso eviscerado (g), demonstrando dimorfismo sexual durante o desenvolvimento em ambas as espécies.Os tamanhos estimados de maturação para machos e fêmeas, respectivamente, foram iguais a 46,1 e 44,7 cm para *S. acuta*; e 58,4 e 59,9 cm para *S. bonapartii*. Uma diminuição nos tamanhos de maturidade para ambos os sexos foi observada em *S. acuta* e fêmeas de *S. bonapartii* da costa Sul do Brasil, com relação a estudos realizados há 30 anos.

Key words: Endemic, Maturity, Morphometric, Oviparity, Reproduction.

Introduction

The genus *Sympterygia* Müller & Henle, 1837 (Chondrichthyes, Rajiformes) constitutes a taxonomically stable clade within the Suborder Rajoidei and includes four neotropical oviparous skate species: *S. lima* (Poeppig, 1835), *S. brevicaudata* (Cope, 1877), *S. acuta* (Garman, 1877) and *S. bonapartii* (Müller & Henle, 1841) (McEachran, 1982; Ebert & Compagno, 2007). The latter two are endemic to the shelves of the western South Atlantic Ocean shelves (Figueiredo, 1977; McEachran & Aschliman, 2004) and represent an important economic resource in their whole distribution area (Mabragaña *et al*., 2002; Menni & Stehmann, 2000; Paesch & Domingo, 2003). In southern Brazil (south and southeast), *S. acuta* and *S. bonapartii* complete their life cycle in the inner-continental shelf waters (De Queiroz, 1986; Vooren, 1997; Vooren *et al.,* 2005).

Like other elasmobranchs, the rajoids present relatively low fecundity, late sexual maturity and high longevity. These characteristics, together with fish exploitation, have led to population decreases worldwide, including cases of local extinction (Dulvy *et al.,* 2000; Dulvy & Reynolds, 2002; Iglésias *et al.*, 2009). The bignose fanskate *S. acuta*, is (globally) considered by the IUCN Red List of Threatened species as "Vulnerable". On the other hand, the smallnose fanskate, *S. bonapartii*, is classified as "Data Deficient" (Massa & Hozbor, 2004; Massa & Lamilla, 2004). However, due to the high fishing pressure to which both species are subject, the mentioned categories may change regionally in a short-term period.

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Studies on the reproductive biology, size-at-maturity and abundance of *S. bonapartii* were carried out in the coasts of Argentina and Uruguay over the last decade, demonstrating an increasing concern for the fisheries effects upon their populations, as well as the need for biological data (especially on reproduction) in order to ensure their sustainable exploitation (Mabragaña *et al*., 2002; Oddone & Velasco, 2004).

De Queiroz (1986) analyzed the reproductive biology of *Sympterygia* spp. in the southern Brazilian shelf. Apart from these data being part of an unpublished thesis, they were collected in the period 1981-1984, *i.e.*, almost 30 years ago. Moreover, it is a well-known fact that in the reproductive parameters, such as the size-at-maturity, may change in the rajoids due to the pressure of excessive fishing (Walters & Martell, 2004).

It is, therefore, necessary, to provide an updated study on the reproductive biology of genus *Sympterygia* in southern Brazil, in order to guide the management decisions needed for their conservation, including measures such as closed areas and minimum landing sizes. The aim of this study was , thus, to provide updated information on the reproductive parameters of *S. bonapartii* and *S. acuta* in the southernmost continental shelf of Brazil between Conceição (31° 43'S) and Chui (33°45'S).

Material and Methods

Samples of *Sympterygia* spp. were obtained from two sources: one-day-long research cruises aboard the research vessel "Larus" (Instituto de Oceanografia, Universidade Federal de Rio Grande), done in May, June, July and August 2011; and two commercial fishing trips carried out along the coast of Rio Grande do Sul State (southern Brazil), from September 22nd to 30th 2011 and from January 30th to February 10th 2012. The studied area was located between the latitudes of 34°28'Sand 31°29'S at depths between 15 and 142 m (Fig. 1). Voucher specimens of *S. acuta* (SA001C; SA002C) and *S. bonapartii* (SB003C; SB004C) were stored in the fish collection of the Laboratory of Morphology and Histology, ICB / FURG, Brazil. A total of 92 males and 125 females of *S. bonapartii* and 17 males and 28 females of *S. acuta* were obtained and analyzed. Of each specimen, the following data were registered: total length (TL) from the snout to the tip of the tail, the width of the disc (DW) measured as the length between opposite pectoral fin tips, total weight (TW), eviscerated weight (EW), liver weight (LW) and gonad weight (GW) (measured values in cm and g, respectively) (*sensu* Oddone *et al.* 2007a).

For males, the clasper and clasper gland length, the number of alar thorns and rows of alar thorns were recorded. Alar thorns considered as ''developing'' were registered as a circular light mark on a darker epidermis, being evident laterally on the dorsal region of both pectoral fins (*sensu* Oddone & Vooren, 2005) The calcification of the clasper was manually assessed and classified as "flexible" or "rigid". From females, the height of the oviducal glands, according to Serra Pereira *et al.* (2011), the number of vitellogenic follicles larger than 1 cm (Mabragaña *et al*., 2002) and smaller than 1 cm (Díaz Andrade *et al*., 2011), the uteri width, diameter and the colour of the largest ovarian follicle, as well as the presence or absence of egg capsules in the uteri and in the cloacal region

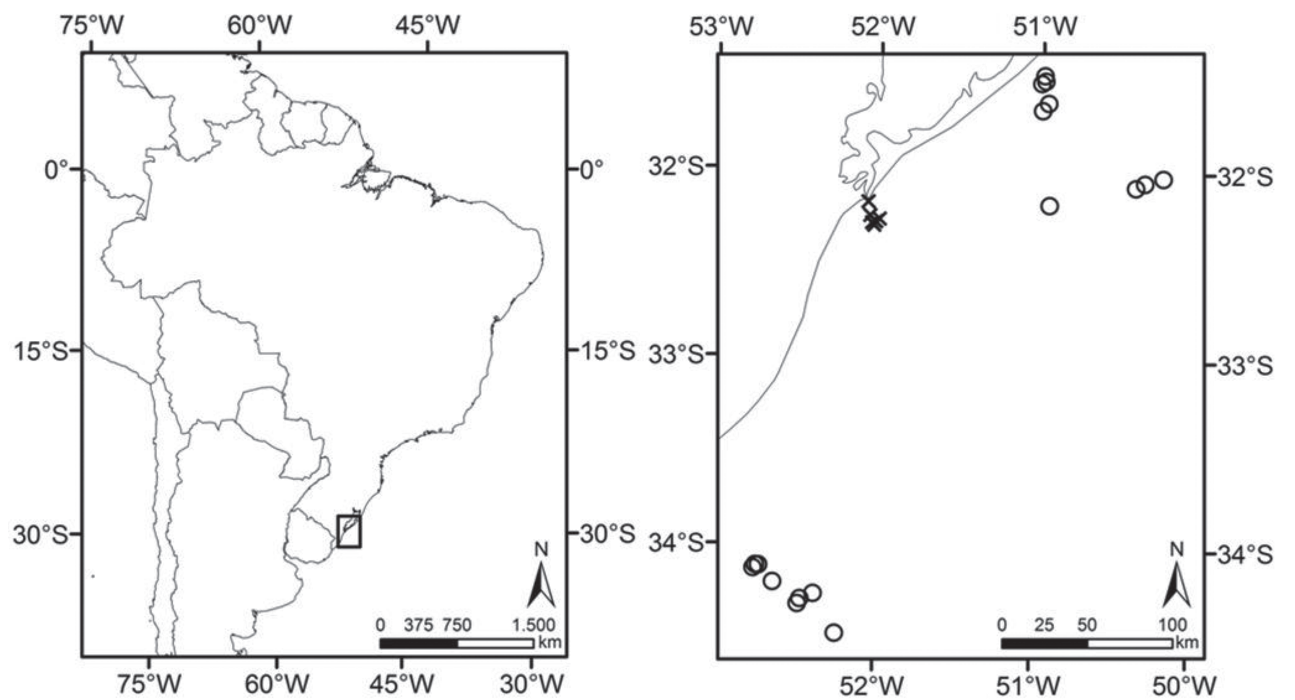


Fig. 1. Map of the study area. Left map represents South America. The square is a detail of the study area. The right map represents south of Brazil indicating the trawling stations where the specimens of *Sympterygia* were captured; research cruises of the research vessel "Larus" (triangles) and commercial bottom trawl fishing trips (circles).

were registered (Oddone & Vooren, 2005). According to the development of the above-mentioned reproductive structures in relation to TL, the individuals were classified in three maturity categories: immature, adolescent and mature (Oddone *et al.,* 2007a).

Morphometric measurements of both species and sexes were compared. Total length-width disc, total length-total weight and total length-eviscerated weight were log-transformed to become linear and the F-test was used to establish comparisons (Souza, 1998). In this respect, a covariance analysis was applied, where the variable "sex" was a factor. We verified if the curves fitting separately substantially reduced the sum of the squared residuals. The logistic equation was used to estimate the size of sexual maturity, as

$$PTL=1/\left(1-e^{(a+bTL)}\right)$$

where, PTL is the fraction of mature individuals for each class of TL, a and b are the model parameters. The TL_{50}, or the size at which 50% of the population is mature, is given by the ratio a/b (Restrepo & Watson, 1991). As the relationships between clasper length and TL and clasper gland length and TL followed a sigmoid-shaped curve, the logistic equation was also fitted in order to estimate the inflexion point of these relationships, and therefore assess the adolescent phase through these variables. In using parametric/non-parametric tests, normality and homogeneity of variance of the variables were tested by Lilliefors' and Levene's tests, respectively. Parametric comparisons were performed using the Student t-test, which was applied to compare the mean number of alar thorns and the mean number of rows of alar thorns and to assess the statistical significance of the differences between the number of ovarian follicles in mature females and egg-bearing females (Sokal & Rohlf, 2012). Reproductive parameters were expressed in terms of mean and standard deviation as mean ± S.D. In all cases "n" is the size of the sample. A level of significance of 0.05 was considered for all the applied tests.

Results

Morphometric relationships in *Sympterygia bonapartii* and *S. acuta*. Total length of male *S. bonapartii* ranged between 47.2 cm and 76.8 cm. Immature males had TLs of 47.2-62.5 cm (mean=56.12; SD=4.41; n=14); adolescents of 55.0-71.6 cm (mean=61.38; SD=4.78; n=19) and in mature specimens TL varied from 58.6 to 76.8 cm (mean=65.46; SD=4.39; n=59). The relationship between TL and DW in males was linear (Fig. 2a) (Table 1).

Total length of female *S. bonapartii* varied between 30.8 and 79.1 cm. Values in immature females varied from 30.8 to 67.5 cm (mean=56.2; SD=12.55; n=17). Adolescents had total length ranging from 36.8 to 73.3 cm (mean=65.1; SD=9.99; n=12). In matures females, TL varied from 58.0 to 79.1 cm (mean=68.8; SD=4.20; n=96) and egg-bearing females had TL ranging from 68.0 to 71.2 cm (mean=69.3; SD=1.35; n=4). The relationship between TL and DW in females was linear (Fig. 2a) (Table 1).

According to F-test results, significant differences were detected between sexes for to the relationship TL-DW, TL-TW and TL-EW for *S. bonapartii* (Table 1, Fig. 2a-c).

Total length of male *S. acuta* varied from 29.5 to 56.5 cm. The values of TL for immature individuals ranged between 29.5 and 47.0 cm (mean=41.5; SD=8.19; n=4). Adolescents ranged from 43.8 to 46.5 cm TL (mean=45.15; SD=1.90; n=2). Mature specimens had TLs varying between 47.0 and 56.5 cm (mean=50.25; SD=2.70; n=11). The relationship between TL and DW in males was linear (Fig. 2d) (Table 1).

In female *S. acuta*, TLs ranged between 21.0 and 60.0 cm. Immature females had TLs of 21.0-51.4 cm (mean=33.07; SD=11.08; n=7); adolescents of 48.9-52.7 cm (mean=50.76; SD=1.46; n=5); and mature specimens of 49.6 to 60.0 cm

Table 1. Relationships analyzed for *S. bonapartii* and *S. acuta*. TL=total length (cm), DW=disc width (cm), TW=total weight (g), EW=eviscerated weight (g) for males (M) and females (F) with the respective potential regression equation; correlation coefficient (r) and sample number (n).

Curve	Sex	Equation	r	n	F-test results
S. bonapartii					
TL-DW	M	DW=2.213+0.6252*TL	0.911	92	F=3.106;d.f.=(2,213);p<0.001
	F	DW=1.921+0.6488*TL	0.890	125	
TL-TW	M	$TW=0.019968TL^{2.71552}$	0.852	87	F=3.314;d.f.=(2,203);p<0.001
	F	$TW=0.043169TL^{2.5428}$	0.745	120	
TL-EW	M	$EW=0.022659TL^{2.66553}$	0.879	87	F=3.174;d.f.=(2,203);p<0.001
	F	$EW=1.30685TL^{2.95601}$	0.743	120	
S. acuta					
TL-DW	M	DW=2.275+0,5069 *TL	0.956	17	F=4.449;d.f.=(2,39);p=0.015
	F	DW=1.295+0.5181*TL	0.970	26	
TL-TW	M	$TW=0.901592TL^{2.15908}$	0.922	17	F=3.438;d.f.= (2,41);p=0.006
	F	$TW=0.041639TL^{2.43972}$	0.754	28	
TL-EW	M	$EW=0.002462TL^{3.1534}$	0.883	17	F=3.461;d.f.= (2,41);p=0.006
	F	$EW=0.048513TL^{2.36878}$	0.761	28	

(mean=53.65; SD=3.08; n=11). Egg-bearing females had values between 47.7 and 58.0 cm TL (mean=53.14; SD=3.74; n=5). The relationship between TL and DW in females was linear (Fig. 2d) (Table 1). In *S. acuta* the relationships between TL-DW, TL-TW and TL-EW showed significant differences (Table 1, Fig. 2 d-f).

Sexual development of male *Sympterygia bonapartii*. The clasper length in immature individuals varied from 3.8 to 11.3 cm (mean=8.73; SD=2.16; n=14). Adolescents had clasper length ranging from 9.5 to 15.4 cm (mean=12.26; SD=1.73; n=19) and in mature males these values varied between 13.5 and 18.1 cm (mean=15.53; SD=1.05; n=59). The onset of clasper

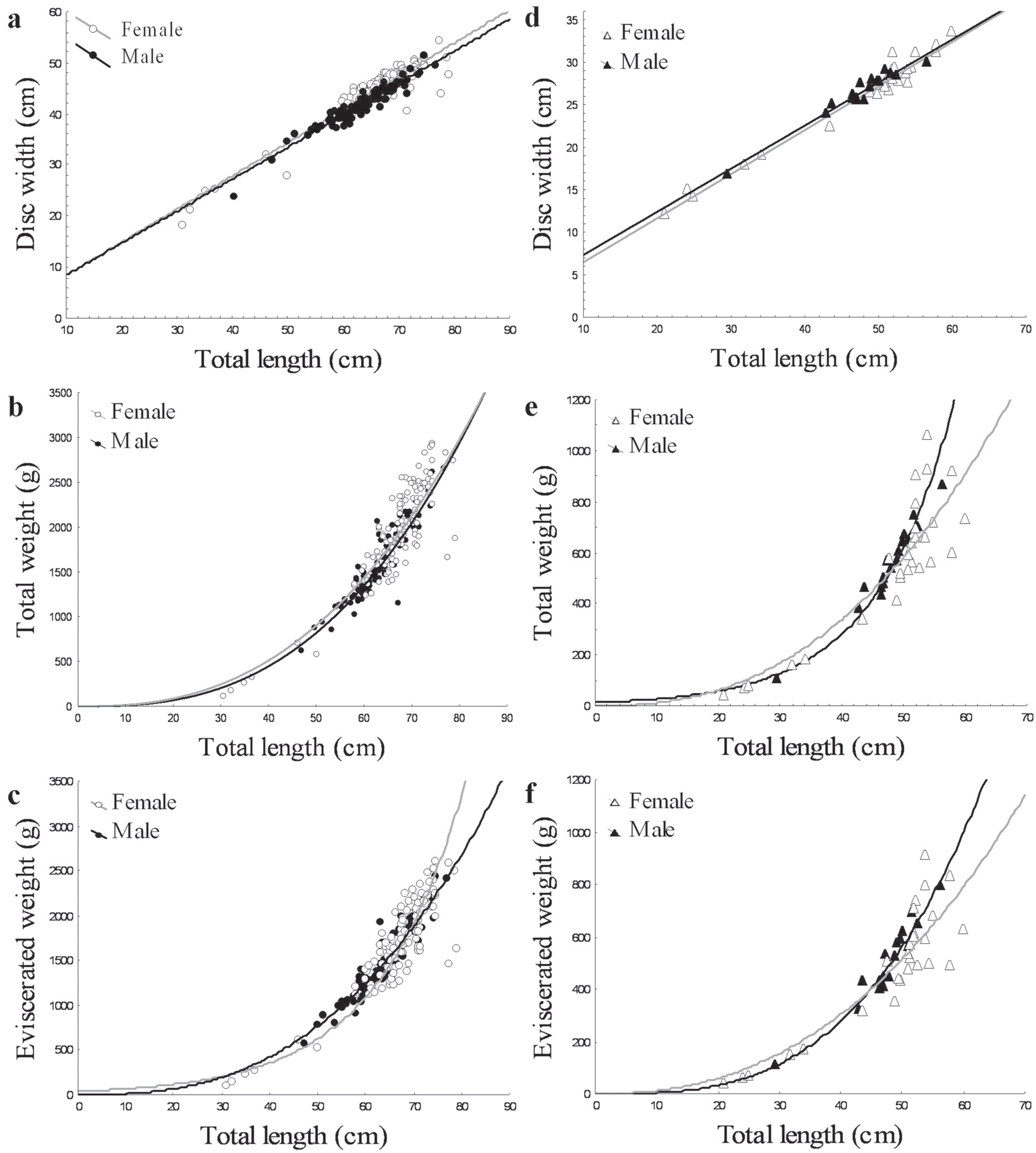


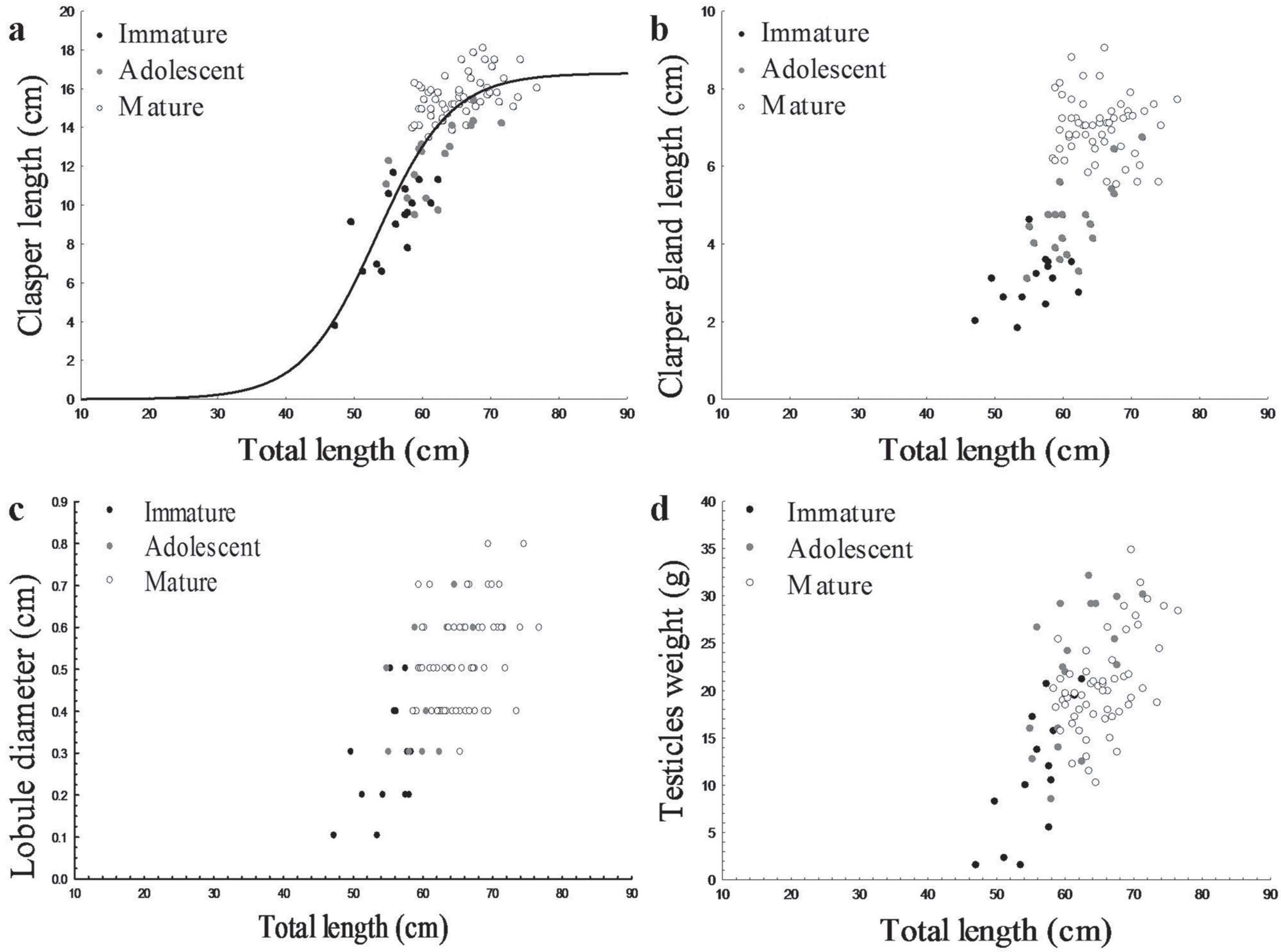
Fig. 2. *Sympterygia bonapartii* (circles) with relationship between total length (cm) and (a) disc width (cm), (b) total weight (g) and (c) eviscerated weight (g) (by sex). *Sympterygia acuta* (triangles) with relationship between total length (cm) and (d) disc width (cm), (e) total weight (g) and (f) eviscerated weight (g).

calcification was observed at TL=55.2 cm. The inflexion point of the clasper length-TL relationship was estimated to be at 53.2 cm (r=0.81; n=92) (Fig. 3a).

The clasper gland length in immature individuals ranged between 1.8 and 3.6 cm (mean=2.94; SD=0.59; n=14). In adolescents this length ranged from 3.1 to 6.7 cm (mean=4.63; SD=0.94; n=19) and in mature individuals from 5.5 to 9.0 cm (mean=6.99; SD=0.77; n=59) (Fig. 3b). The inflexion point of the clasper gland-TL relationship was estimated at 57.3 cm (r=0.60; n=92).

Testicular lobules became macroscopically visible at TLs of 47.2 cm onward. Immature individuals had testicular lobules with diameters varying between 0.1 and 0.5 cm (mean=0.3; SD=0.14; n=14).The adolescent lobule diameter ranged between 0.3 and 0.7 cm (mean=0.47; SD=0.12; n=19). Mature males had testicular lobules with diameters between 0.3 and 0.8 cm (mean=0.52; SD=0.12; n=59) (Fig. 3c).

Testicles weight varied from 1.5 to 21.1 g (mean=11.34; SD=6.91; n=14) in immature specimens, from 8.4 to 32.1 g (mean=22.14; SD=7.19; n=19) in adolescents and from 10.2 to



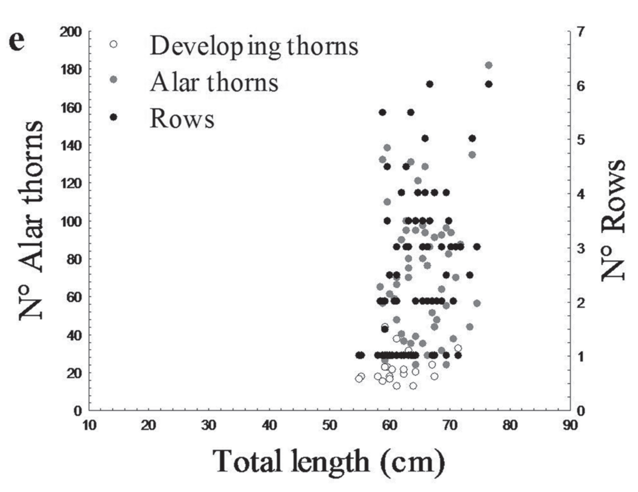


Fig. 3. *Sympterygia bonapartii.* Relationship between total length (cm) and (a) clasper length (cm), (b) clasper gland length (cm), (c) diameter of the testicular lobule (cm), (d) testicles weight (g) and (e) total number of alar thorns (left Y axis) and total number of rows of alar thorns (right Y axis).

34.7 g (mean=20.45; SD=4.96; n=59) in mature males (Fig. 3d).

The number of alar thorns rows varied from 1 to 6 on each pectoral fin. No significant difference was detected between left and right pectoral fins regarding the mean alar thorn rows number (mean=2.49, SD=1.38, n=77; mean=2.46, SD=1.47, n=77, respectively) (t=0.11; d.f.=76; p=0.91). The number of alar thorns ranged between 6 to 88 on the right fin and from 6 to 94 on the left one (mean=30.64, SD=19.84, n=77; mean=30.57, SD=20.60, n=77, respectively) with no significant differences between fins (t=0.019; d.f.=76; p=0.98). The number of developing thorns varied from 6 to 24 and from 6 to 20 for right and left pectoral fins, respectively (mean=11.05, SD=4.22, n=20; mean=10.75, SD=4.12, n=20, respectively) (Fig. 3e). Differences between fins were not significant (t=0.22; d.f.=38; p=0.82).

Immature individuals with developing thorns had TLs smaller than 62.5 cm. An adolescent individual with TL of 50.0 cm showed developing thorns, which were also recorded in two mature specimens with TLs of 59.6 and 61.3 cm respectively (Fig. 3e).

The size at which 50% of the population is mature, TL_{50} was estimated at 58.4 cm (r=0.98; n=92) for male *S. bonapartii* (Fig. 4).

Sexual development of male *Sympterygia acuta.* The measures of clasper length in immature individuals of *S. acuta* varied 0.9 to 3.7 cm (mean=2.8; SD=1.29; n=4); in adolescents from 7.6 to 9.4 cm (mean=8.5; SD=1.27; n=2); and in mature individuals from 7.3 to 13.0 cm (mean=9.5; SD=1.92; n=11). The onset of calcification occurred when males attained a TL of 43.8 cm. The inflection point of the curve clasper length vs. Total length was 43.9 cm (r=0.69; n=17) (Fig. 5a).

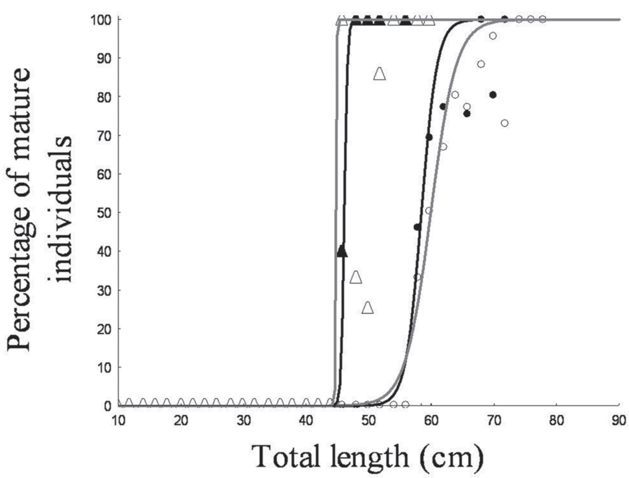


Fig. 4. Percentage of mature individuals by total length class for males and females *Sympterygia acuta* (black line) and *S. bonapartii* (grey line). Empty triangles represent male *Sympterygia bonapartii* and full triangles, male *S. acuta.* Empty circles represent female *S. bonapartii* and full circles, female *S. acuta*.

Individuals with TL between 43.8 and 52.5 cm, had values of clasper gland length between 2.3 and 5.2 cm. (Fig. 5b).

The testicular weight of immature individuals varied between 0.5 and 2.0 g (mean=1.39; SD=0.61; n=4). The testicles weight in adolescents varied between 5.0 and 10.0 g (mean=7.5; SD=3.53; n=2). Mature males had testicular weights between 6.0 and 13.9 g (mean=10.79; SD=2.24; n=11) (Fig. 5c).

The number of alar thorns rows varied between 4 and 6, on both right and left pectoral fins (mean=5, SD=0.63, n=11; mean=4.9, SD=0.70, n=11, respectively) with no significant differences between fins (t=0.31; d.f.=10; p=0.75). The number of alar thorns varied from the 16 to 75 on the right and from 14 to 68 on the left pectoral fin (mean=29.36, SD=21.03, n=11; mean=27.91, SD=18.8, n=11, respectively) (Fig. 5d) with no significant difference (t=0.17; d.f.=10; p=0.86) (Fig. 5d). The TL_{50} for *S. acuta* males, was estimated at 46.1 cm (r=0.99; n=17) (Fig. 4).

Sexual development of female *Sympterygia bonapartii.* Females with ovary weights between 1.2 and 10.2 g, were immature with no vitellogenic activity (mean=6.8; SD=2.81; n=13). Adolescent females had white and yellow ovarian follicles, with gonad weight ranging from 0.5 to 16.2 g (mean=8.7; SD=3.87; n=12). Mature females had ovaries weighing between 4.8 and 108.5 g, bearing only yellow vitellogenic follicles (mean=29.2; SD=18.69; n=92). Vitellogenic follicles in egg-bearing females occurred with corresponding ovarian weights between 28.1 and 72.2 g (mean=46.9; SD=18.61; n=4) (Fig. 6a).

White follicles diameter ranged between 0.1- 1.0 cm (mean=0.4; SD=0.27; n=17) and were recorded in immature individuals and adolescents of up to 72.5 cm TL. The onset of vitellogenesis occurred when follicles attained a diameter of 0.4 cm. The smallest female with vitellogenic yellow follicles was 36.8 cm TL. The diameter of vitellogenic follicles in mature females varied between 0.4- 4.0 cm (mean=2.0; SD=0.83; n=97). Egg-bearing females had follicles with a diameter between 2.3 and 2.9 cm (mean=2.6; SD=0.25; n=4) (Fig. 6b).

Mean values for uterus width in females varied among all the maturity stages analyzed. Immature individuals had uteri ranging from 0.2 to 2.2 cm wide (mean=1.0; SD=0.62; n=17). Meanwhile, adolescent females had uteri with widths of 0.4 to 2.4 cm (mean=1.5; SD=0.55; n=12). In mature individuals, uteri width varied from 1.0 to 3.8 cm (mean=2.5; SD=0.48; n=92). Within this group, egg-bearing females had uteri ranging between 2.1 and 3.3 cm wide (mean=3.8; SD=0.43; n=4) (Fig. 6c).

The Oviducal gland height (OGH) varied from 0.4 to 1.9 cm (mean=1.1; SD=0.47; n=13) in immature females and from 0.4 to 1.9 cm (mean=1.2; SD=0.51; n=10) in adolescents. Mature females had OGHs varying from 1.3 to 3.5 cm (mean=2.1; SD=0.42; n=91), while egg-bearing females had OGHs ranging from 2.1 to 3.3 cm (mean=2.6; SD=0.52; n=4) (Fig. 6d). Ovarian fecundity varied from 9 to 74 (mean=29.81; SD=10.85; n=82). The number of vitellogenic yellow follicles with less than <1

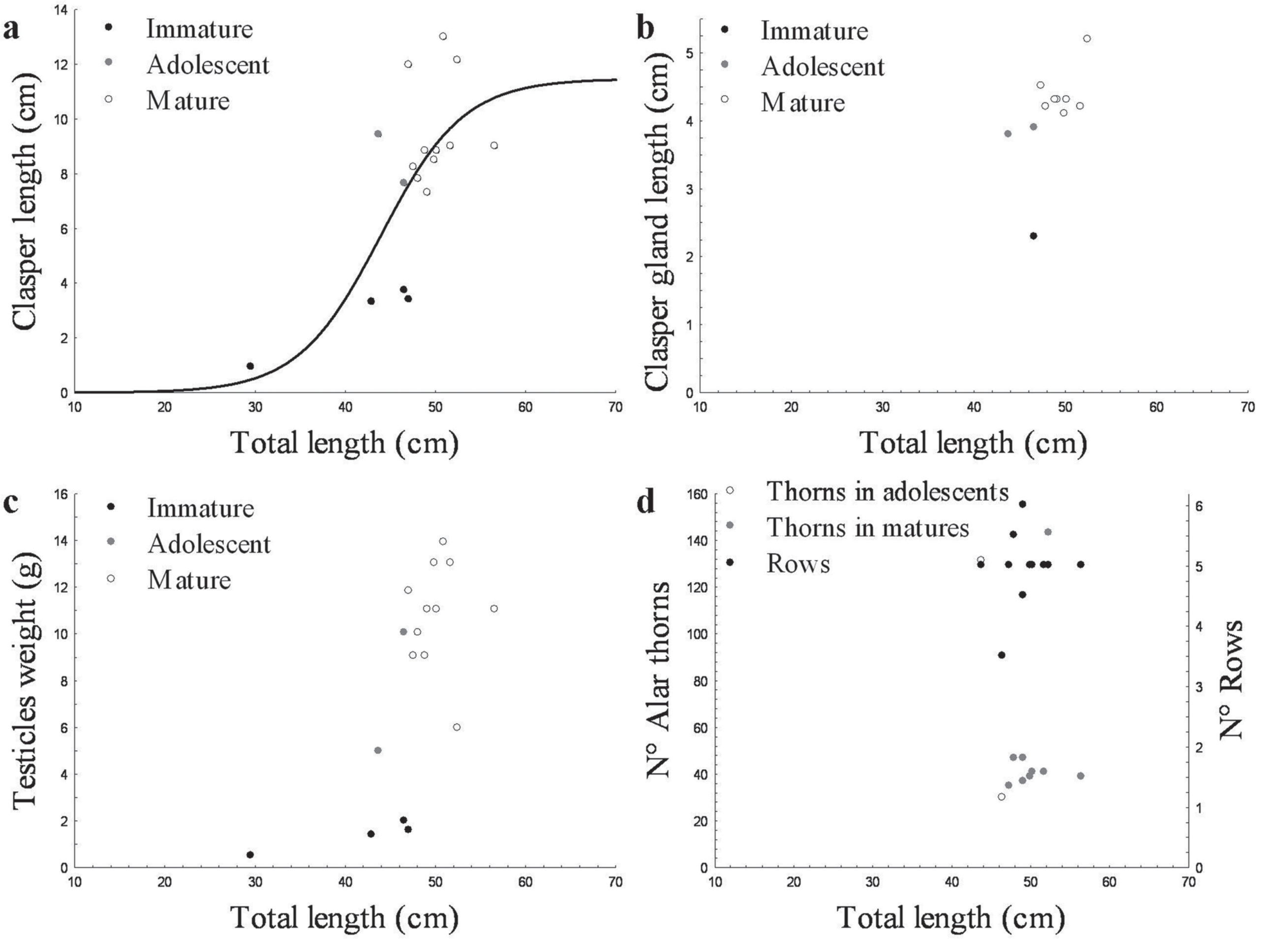


Fig. 5. *Sympterygia acuta.* Relationship between total length (cm) and (a) clasper length (cm), (b) clasper gland length (cm), (c) testicles weight (g) and (d) total number of alar thorns (left Y axis) and total number of rows of alar thorns (right Y axis).

cm in diameter, in individuals classified as adolescent and mature, varied from 4 to 54 follicles (mean=23.16; SD=15.46; n=13). In egg-bearing females, the number of follicles varied from 21 to 35 (mean=29.3; SD=6.02; n=4). No difference was observed, between the number of vitellogenic follicles larger than 1 cm and the number of follicles in egg-bearing females (t-test for independent samples, t=0.10; d.f.=84; p=0.91) (Fig. 6e). The TL_{50} estimate for *S. bonapartii* was 59.9 cm (r=0.99; n=125) (Fig. 4).

Sexual development of female *Sympterygia acuta.* Ovaries of immature females weighted between 0.4 and 3.0 g (mean=1.46; SD= 1.10; n= 5). Adolescents had both white and yellow follicles, with ovaries weighting between 3.8 and 8.5 g (mean=6.04; SD=2.18. n=5). Mature females bore yellow follicles and had ovaries weighting between 8.2 and 35.0 g (mean=21.71; SD=8.96; n=7). Ovaries of egg-bearing females weighted between 20.8 and 40.0 g (mean=33.95; SD=9.07; n=4) (Fig. 7a).Uteri width in immature females varied from 0.1 to 0.6 cm (mean=0.31; SD=0.19; n=7). Adolescent females had uteri width ranging from 0.6 to 2.0 cm (mean=1.38; SD=0.50; n=5). In matures females, uteri width varied from 0.4 to 1.7 cm (mean=0.81; SD=0.55; n=11) and egg-bearing females had uteri width from 2.6 to 3.6 cm (mean=3.08; SD=0.37; n=5) (Fig. 7b).

Immature females with TL up to 51.4 cm had white follicles diameter varying from 0.1 to 0.5 cm (mean=0.20; SD=0.2; n=5). Vitellogenesis was macroscopically evident when follicles attained a diameter of 0.6 cm. The smallest female with vitellogenic yellow follicles was 48.9 cm long. The diameter of vitellogenic follicles in mature females varied between 0.6 and 2.4 cm (mean=1.6; SD=0.51; n=14) and in the egg-bearing females from 1.5 to 2.5 cm (mean=2.04; SD=0.43; n=4) (Fig. 7c).

OGH varied from 0.1 to 0.6 cm (mean=0.41; SD=0.19; n=4) in immature females; from 1.3 to 1.7 cm (mean=1.52; SD=0.17; n=4) in adolescents, and from 2.0 to 3.1 cm (mean=2.48; SD=0.27; n=11) in mature females. Egg-bearing females had OGHs varying from 2.0 to 2.7 cm (mean=2.36; SD=0.28; n=5) (Fig. 7d). The TL_{50} was estimated at 44.7 cm (r=0.90; n=28) for female *S. acuta* (Fig. 4).

Discussion

The relationships TL-DW, TL-TW and TL-EW for *S. acuta* and *S. bonapartii* indicated differences between males and females for both species. Sexual dimorphism in these relationships has been described for *S. bonapartii* (Mabragaña *et al.,* 2002) and for other SW Atlantic rajoids (Mabragaña & Cousseau, 2004; Oddone & Vooren, 2004; Oddone & Amorim, 2007; Orlando *et al.,* 2011).

The relationship TL-DW was sexually dimorphic in *S. bonapartii*, similarly to the congeneric species, *S. lima*, endemic of the coast of Chile, Southeast Pacific Ocean (Lamilla *et al*., 1984).

The TL-DW relationship in *S. acuta* indicated that

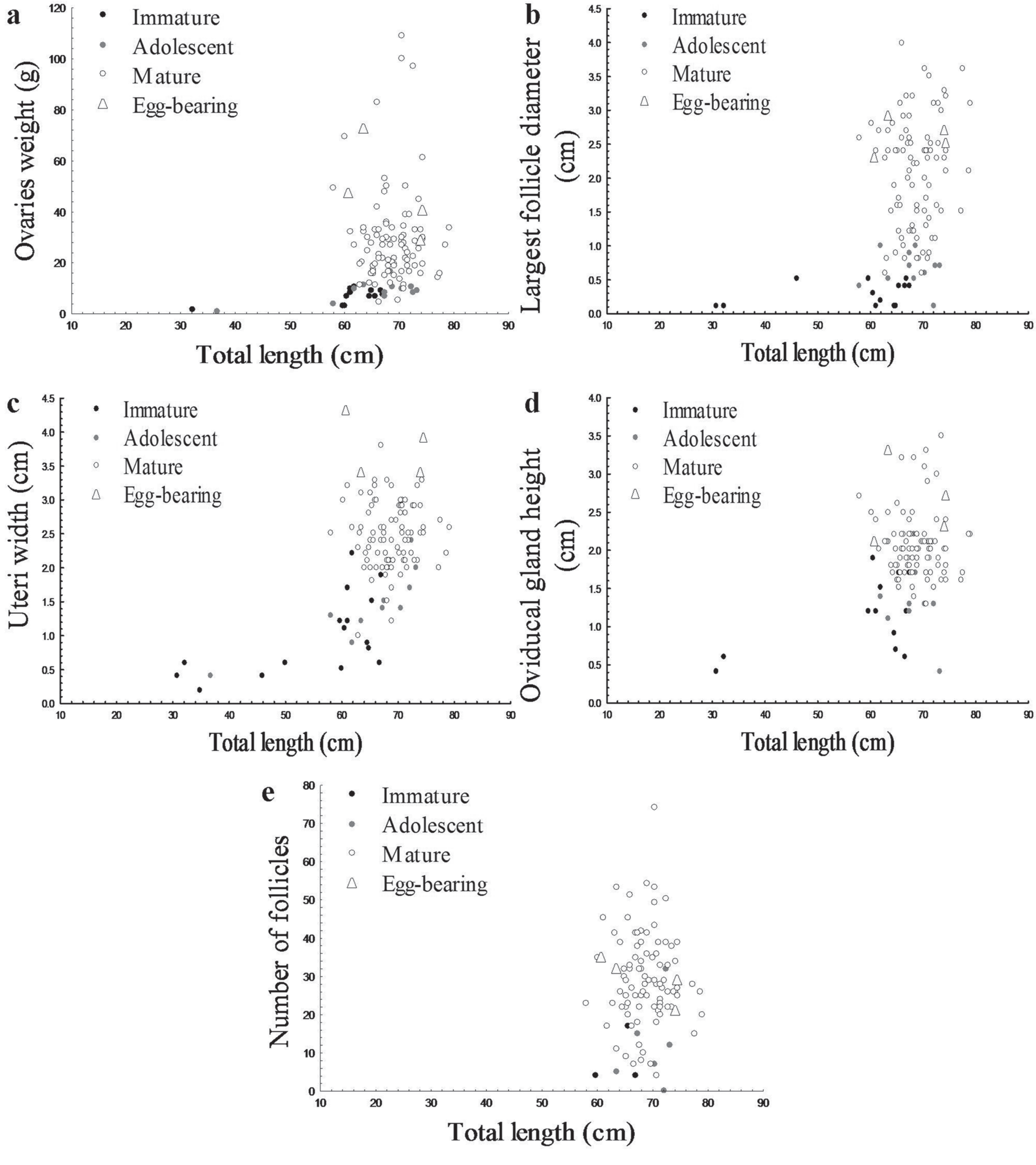


Fig. 6. Sexual development for females of *Sympterygia bonapartii*. Relationship between total length (cm) and (a) ovaries weight (g), (b) diameter of the largest follicle (cm), (c) uteri width (cm), (d) oviducal gland height (cm) and (e) number of follicles.

immature males had higher size when compared with females in this first stage of development. However, in adolescents and mature individuals just the opposite was observed. Such morphometrics differences throughout the ontogeny were also recorded in *Atlantoraja cyclophora* (Regan, 1903) and *Rioraja agassizi* (Muller & Henle, 1841) in southern Brazil (Oddone & Vooren, 2004; Oddone *et al.,* 2007b). On the other hand, the growth pattern in *S. bonapartii* demonstrated that, in this species, females had larger DW than males for any TL considered.

Minimum size at first maturity was similar (or close) for both sexes of *S. bonapartii*. Mabragaña *et al*. (2002) observed similar situation in the same species off Argentina. However, according to Mabragaña *et al*. (2002) different populations might have distinct size at maturity, as it seems to be the case for individuals captured off Argentina (63.5 cm for females and 65.0 cm for males) when compared with those obtained in the present study, off southern Brazil.

The length of clasper in relation to TL in males of *S. bonapartii* described a sigmoid curve, typically observed for the sexual development of rajoids and most elasmobranchs. Studies on other species of the family Arhynchobatidae (Fowler, 1934) demonstrated that the total clasper length and clasper gland length fitted a sigmoid growth curve, at least in the genus *Atlantoraja* and *Rioraja agassizi* (Oddone & Vooren 2005; Oddone *et al*., 2007a; Oddone & Amorim 2008; Oddone *et al*., 2008).This type of logistic growth curves were also observed in four species of the family Rajidae (Blainville, 1816), namely, *Leucoraja ocellata* (Mitchill, 1815), *Leucoraja erinacea* (Mitchill, 1825), *Amblyraja radiata* (Donovan, 1808) and *Malacoraja senta* (Garman, 1885), distributed off the east coast of Canada (McPhie & Campana, 2009).

The number of rows of alar thorns in males of *S. bonapartii* varied between 1 and 6, in agreement with data reported by Mabragaña *et al*. (2002), and also similar to the pattern observed in *A. cyclophora* (Oddone & Vooren, 2005) and *Bathyraja albomaculata* (Ruocco *et al.,* 2006). This sexual character present in males can vary among species, being an important parameter used for maturity staging.

Sympterygia bonapartii showed a high level of overlap among the three stages of sexual development, regarding the following variables: testicle weight, diameter of lobule, clasper gland length, number of rows of thorns and alar thorns. However, mean values of these variables indicate differences

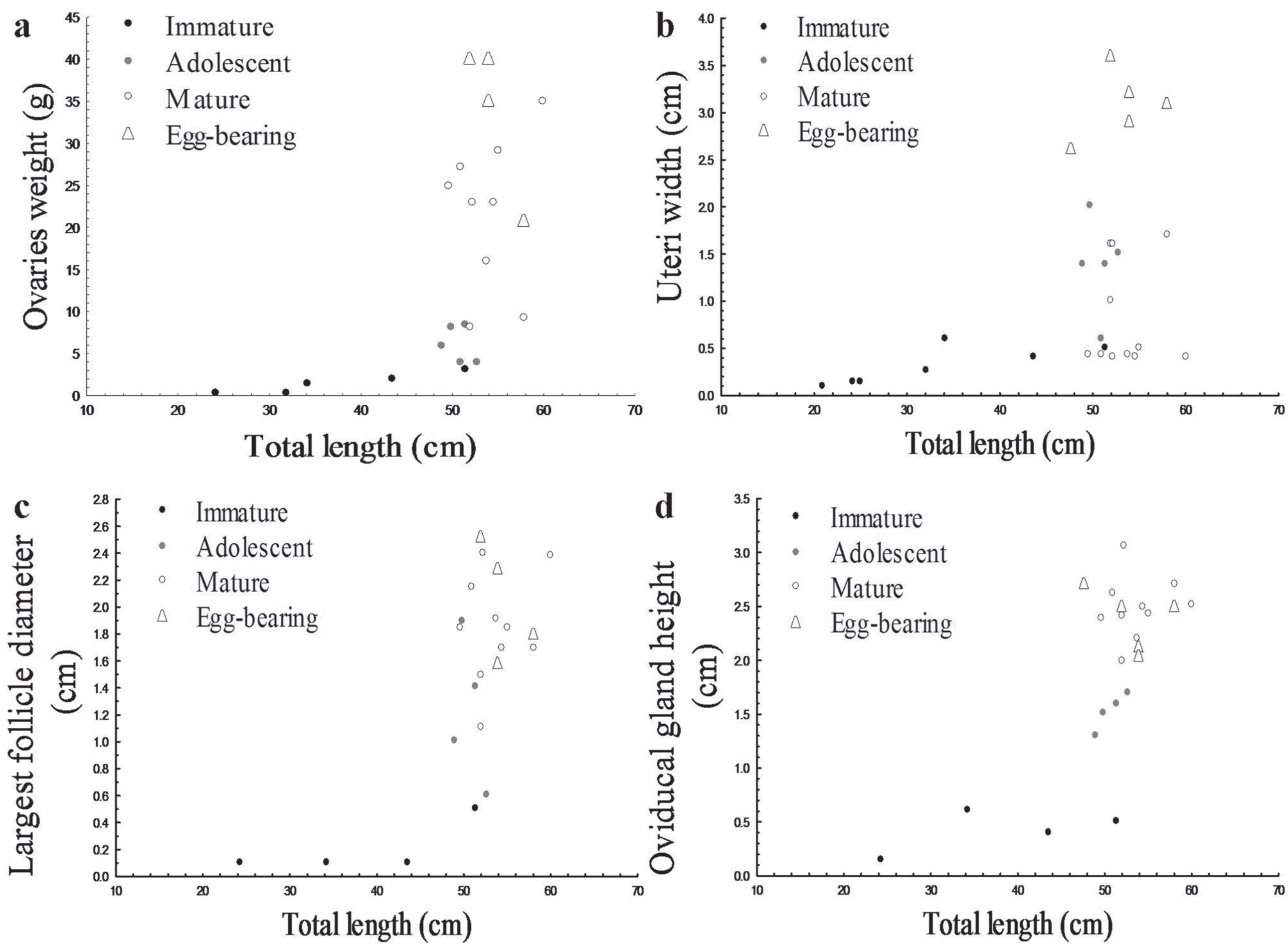


Fig. 7. Sexual development for females of *Sympterygia acuta*. Relationship between total length (cm) and (a) ovaries weight, (b) uteri width (cm), (c) diameter of the largest follicle (cm) and (d) oviducal gland height (cm).

between each stages of sexual development.

Males *S. bonapartii* were found to mature with a TL of 58.4 cm. De Queiroz (1986), however recorded a value of 52 cm for this parameter for southern Brazil, indicating that TL_{50} may have increased in 3.3 % (6.4 cm) over a ~30-year period. However, the differences in the estimated sizes at maturity may also be due to methodological differences (Oddone & Velasco, 2004). De Queiroz (1986) methodology for recording clasper length (based on Hubbs & Ishiyama, 1968) differed from the one used in the present paper, though he used virtually the same reproductive variables for male sexual maturity staging. For male *S. bonapartii*, TL_{50} (58.4 cm) also differed from those calculated from other south western Atlantic areas, such as Argentina (65 cm) and Uruguay (57 cm) (Mabragaña *et al*., 2002; Oddone & Velasco 2004), though the value reported by Oddone & Velasco (2004) off Uruguay (50.0-57.0 cm) was very close to the one obtained in the present study. This similarity may be a consequence of the proximity of the area of the sampled specimens (in the extreme south of Brazil), with the border area between the coasts of Brazil and Uruguay.

In male *S. acuta*, the inflection point of the curve fit for the relationship between clasper length and TL coincided with the value of minimum TL for the clasper calcification. Therefore, the size at maturity based solely on the TL-clasper length relationship could be underestimated. A similar situation was also observed in *S. bonapartii,* demonstrating the importance of the determination record of the clasper calcification degree used for assessing maturity in males of Chondrichthyes.

Adult males of *S. acuta* bore a maximum of 6 rows of alar thorns, similarly to *S. bonapartii*. Other studies have also demonstrated a similarity in this number of maximum of rows of alar thorns, for other species of the same family (Mabragaña *et al*., 2002; Oddone & Vooren, 2005; Ruocco *et al*., 2006).

Contrarily to what was observed for males of *S. bonapartii*, a decrease in TL_{50} corresponding to 3.0 % (5.9 cm) was observed for males of *S. acuta*, when the present data are compared with the value obtained by De Queiroz (1986) for the same region.

Likewise, the ovary weight of mature females of *S. bonapartii* analysed by De Queiroz (1986) varied from 25 to 170 g. For mature specimens of *S. acuta* the same author recorded ovaries weight of 10 to 50 g. When compared to the data found in this study, ovary weights for mature females seem to have decreased since the work done by De Queiroz (1986). Females of both species may have developed at least some degree of reproductive plasticity, reflected in this case in a decrease of gonadal size of mature individuals, which could be a result of fishing mortality in the region during the past decades.

Ovarian follicles larger than 1.0 cm were considered vitellogenic by Mabragaña *et al*. (2002) for *S. bonapartii*. Agreeing with these authors, De Queiroz (1986) stated that vitelogenesis started at 1.0 and 1.5 cm in *S. bonapartii* and *S. acuta*, respectively. However, Díaz Andrade *et al*. (2011) examined ovarian follicles smaller than 1.0 cm in mature females of *S. bonapartii* through the histological analysis of the ovary and concluded that the traditional macroscopic assessment of the follicular vitellogenesis may turn into a subjective parameter of analysis, turning the classification of the development stage difficult.

They demonstrated that vitellogenic follicles with diameters larger than 0.3 cm are characteristic of mature females of *S. bonapartii*, a size that is very close to the diameter of vitellogenic follicles found in the present study (0.4 cm). The analysis performed by Díaz Andrade *et al.* (2009) in females of *S. acuta*, in turn, revealed that, at histological level, vitellogenesis would start at 0.55 cm, a size again similar to the obtained in the present study, where vitellogenesis was macroscopically detected from follicles with diameters of 0.6 cm onward.

Serra Pereira *et al*. (2011) analyzed the relationship of various measures of oviducal gland, including height, width and thickness, in *Raja clavata* (Linnaeus, 1758), in relation to maturity stages (*i.e*., "in development"; "spawning capable"; "actively spawning"), finding differences among stages. Differences in the OGH were also detected in *S. bonapartii* and *S. acuta*, whit their values increasing during sexual development.

The size of maturity obtained for females of *S. bonapartii* and *S. acuta*, when compared with those values reported by De Queiroz (1986) in southern Brazil, indicated a considerable decrease in the size of maturity over the last 30 years, corresponding to 7.0 and 4.4 % (or 10 and 8.3 cm) for female *S. bonapartii* and *S. acuta*, respectively. Such a reduction may be caused by fishing mortality (as well as fishing gear selectivity), which is very intense in the southern region of Brazil. This was demonstrated in other areas by Walters & Martell, 2004; Paesch & Oddone, 2008. The TL_{50} estimates obtained for both sexes of *S. bonapartii* demonstrated differences at regional level. The restricted distribution of the species could indicate the existence of two different populations of *S. bonapartii*, the first occupying the higher latitudes of the south western Atlantic Ocean, in the coast of Argentina, and a second one composed by individuals that transit between the coastal zones of Uruguay and southern Brazil. This could be attributed to the transport of water masses from the Brazil Current and the Malvinas Current, accentuated mainly during summer and winter, respectively (Miloslavich *et al*., 2011).

The genus *Sympterygia* from the south-western Atlantic may have suffered a decline as a consequence of strong fishing pressure upon their populations over the decades. Characteristics related to reproduction such as the maturity size may change in oviparous skates that are affected by fishing (Ebert *et al.,* 2008; Paesch & Oddone, 2008; Orlando *et al*., 2011). As it is the case of *Sympterygia* spp., it is essential to have current data on reproduction. Furthermore, future research should also be focused on the reproductive cycle both temporally and spatially. Significant decrease in the size at maturity as a result of fishing pressure was documented for *Dipturus chilensis* (Paesch & Oddone, 2008) in the south-western Atlantic Ocean.

Data on the reproductive biology of the genus *Sympterygia* endemic to the south-western Atlantic Ocean presented in this study, may provide tools for the evaluation of the stocks, in order to properly conserve and manage the populations of *Sympterygia* spp. that are used for human consumption and exploited unrestrictedly as fishery resources.

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