

**Sexual development and reproductive cycle of the Eyespot skate
Atlantoraja cyclophora (Regan, 1903)
(Chondrichthyes: Rajidae: Arhynchobatinae), in southeastern Brazil**

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*Specimens of *Atlantoraja cyclophora* were collected monthly from commercial fishing landings at Guarujá, São Paulo State, Brazil, from March 2005 to April 2006 at depths between 10 and 146 m. Males ranged from 13.3 to 58.5 cm TL ($n = 396$). Both the smallest mature male and the largest immature male were 47.0 cm long. Males' size-at-50% maturity was calculated to be 46.3 cm. Females ranged from 11.5 to 68.0 cm ($n = 401$). The smallest mature and the largest immature female were 51.6 and 53.0 cm long respectively. For the females, size-at-50% maturity was calculated to be 53.2 cm. In the males the hepatosomatic and gonadosomatic indices varied between 0.48 (August) and 3.54 (November) and between 0.15 (November) and 1.45 (June) respectively, with no significant variation for the fourteen-month period. In the females the hepatosomatic and gonadosomatic indices varied from 1.55 and 6.30 3.54 (both for April 2006) and from 0.08 (December) to 4.41 (October) respectively, with no significant difference among months. Egg-bearing females were found in all months with proportions varying from 0.03 (March) to 0.67 (April). Both males and females undergo an annual cycle, with slight seasonal variations in reproductive activity and a peak in the proportion of egg bearing females between April and July.*

Key words: clasper, elasmobranchs, follicles, egg-bearing, gonads, reproduction, sexual resting period

INTRODUCTION

The genus *Atlantoraja* Menni 1972 is endemic to the south-western Atlantic Ocean (McEACHRAN & ASCHLIMAN, 2004). *Atlantoraja cyclophora* (REGAN, 1903) occurs from Cabo Frio (Rio de Janeiro State) to Argentina, being commonly found in coastal areas down to 150 m depth (FIGUEIREDO, 1977). However, ODDONE & VOOREN (2004) observed that the species occurs at depths of down to 300 m off southern Brazil, though in south-eastern Brazil, the species is commonly caught in the range of 10 to 130 m depth (ODDONE & AMORIM, 2007).

In southern Brazil, *A. cyclophora* occurs throughout the year without seasonal fluctuations in abundance and completes its life cycle in the area (VOOREN, 1998). ODDONE & VOOREN (2004) observed no significant differences in the frequency of occurrence and abundance (CPUE) between latitudes, depth and season and no clear relation between abundance and depth, temperature or salinity, with coexisting males and females virtually in the same proportion.

With regard to reproductive aspects, ODDONE *et al.* (2004) provided a description of the egg capsule of *A. cyclophora* and ODDONE & VOOREN (2005) described the reproduction of *A. cyclophora* in southern Brazil for a two-season period, from depths between 100 and 300 m, providing estimates of the size-at-maturity for both sexes for that area.

Differences in size-at-maturity for a given species in different geographical areas have been reported for skates. TEMPLEMAN (1987) noted that sexual maturity of the thorny skate, *Raja radiata* occurred at a relatively small size (44-50 and 44-47 cm TL for males and females, respectively) off northern Iceland and western Greenland, off Baffin Island and Labrador, on the Northeast Newfoundland Shelf and in the Gulf of St. Lawrence although considerably larger sizes were found (68-83 and 65-74 cm TL for males and females, respectively) on the Grand Bank and St. Pierre Bank. The same author noted that in areas where skates attained sexual maturity with small lengths, the maximum skate lengths were typically small and that

in areas where skates matured with much greater lengths, the maximum observed lengths were considerably larger.

For *Rioraja agassizi*, endemic to the SW Atlantic, COLONELLO *et al.* (2007) estimated size-at-maturity for the area situated between southern Brazil and northern Argentina, at 47 cm for males and 52 cm for females, whereas ODDONE *et al.* (2007) reported contrasting smaller sizes of 32 and 40 cm, respectively, for southeast Brazil.

Specimens of *Atlantoraja* spp. are commonly landed and commercialised in Santos and Guarujá, especially the largest ones (ODDONE, pers. obs.), and intensive fisheries in the south-western Atlantic have led to overexploitation of several species of demersal elasmobranchs (VOOREN & KLIPPEL, 2005). Fishing pressure upon *A. cyclophora* is known to be intense across the species' range in southern Brazil where demersal trawl fisheries operate and skates are landed as part of multi-species fisheries. No conservation measures exist in Brazil, in spite of the fact that the species is considered by the IUCN Red List of Threatened Species as 'vulnerable' (HOZBOR *et al.*, 2004).

The aim of this paper is to describe the sexual development of males and females of *A. cyclophora* and to provide an estimation of the size-at-maturity in the area of southeastern Brazil, in order to compare this parameter with the estimations for southern Brazil, and finally, to assess the reproductive cyclicality of this species on an annual basis, which remains unknown to date.

MATERIAL AND METHODS

Data collection and sampling methods

Specimens of *A. cyclophora* were collected monthly from commercial landings at Guarujá, São Paulo State, Brazil, from March 2005 to April 2006 by eight fishing vessels that regularly provided (once or twice a month) samples of this species. The study area was situated between latitudes 23°37'S and 27°40'S, at depths between 10 and 146 m (Fig. 1).

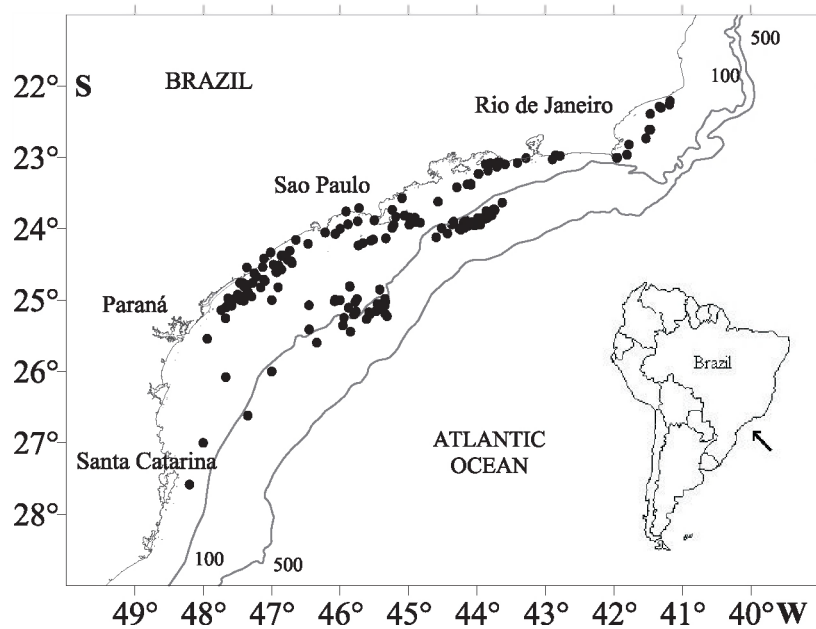


Fig. 1. Map of the study area, southeast Brazil, southwestern Atlantic Ocean. Symbols represent the total number of fishing hauls (when registered by fishermen) in the area where samples of *Atlantoraja cyclophora* were collected

Specimens were measured to the nearest millimetre as total length (TL) and weighed as total (M_T) and gutted (M_G) weight (g). Gonad and liver weight (g) were recorded in both sexes. Electronic scales used had 1 and 5 g precision. For weighting material of less than 1.0 g, a precision scale was used.

Reproductive data recorded

Reproductive variables recorded in males were: clasper and clasper gland length (cm), number of alar thorn rows, number of alar thorns per row, number of developing thorns (*sensu* ODDONE, 2003), diameter of the largest testicular lobe (cm) and testis weight (g). Clasper length was measured *sensu* COMPAGNO (1984). Clasper calcification degree was manually registered, with the clasper classified as 'rigid' or 'flexible', for maturity assessment.

In females the variables recorded were: nidamental gland and uterus width (cm), diameter (cm) and colour of the largest ovarian follicle; number of follicles of the mature group, *i.e.* ovarian follicles (with minimum diameter varying among species) already containing yolk (FITZ & DAIBER, 1963), characterized by a bright

orange colour in this species and the presence of egg capsules in the uteri and/or *cloaca*. The volume (ml) of the largest ovarian follicle and the ovulated and encapsulated oocyte was measured in some specimens to provide an estimation of the ovulatory size. Egg capsules in formation, *i.e.* those cases in which about 5-70% of the egg capsule was already formed, were registered.

Data analysis

Gonadosomatic and hepatosomatic indices were calculated as: $GSI = (\text{gonad weight} / M_G) * 100$ and $HSI = (\text{liver weight} / M_G) * 100$, respectively.

Specimens were grouped into three categories; *i.e.* immature, juvenile and mature, according to criteria defined by ODDONE *et al.* (2007) for *Rioraja agassizi*. A logistic curve was fitted to the relationship between the fraction of mature males or females as a function of TL: $P_{TL} = 1 / (1 + e^{-(a+bTL)})$, where P_{TL} is the fraction of mature individuals in the length class TL, and a and b are the model parameters. With this model mean size-at-maturity, TL_{50} , which represents the body size at which 50% of the skates are mature, was estimated by a/b , (RESTREPO & WAT-

SON, 1991). Maturity estimations were corrected for downward rounding of total length measurements according with FRANCIS & Ó MAOLAGÁIN (2000), by adding 0.5 cm.

As the monthly sample size of egg-bearing females and mature females was small and unequal, the application of a statistical test was not possible. Therefore, to compare the percentage of egg-bearing females per month, the standard deviation of the percentages corrected by the sample fraction (COCHRAN, 1977) was calculated as: ' $p \pm sp$ ' where p = monthly proportion of egg-bearing females calculated as the proportion of egg-bearing females (n) relative to the total of mature females (N) for a given month; and sp . (percentage standard deviation) = $\sqrt{(1-f) * \sqrt{p*q/n-1}}$, with $q = 1-p$ and $f = n/N$. Because of the low number of egg bearing females by month in March, samples from March 2005 and March 2006 were summed in order to consider the variation in the number of egg bearing females on an annual basis.

Statistical support

In using parametric/non-parametric tests, normality and homogeneity of variance of the variables were tested by Lilliefors' and Levene's tests, respectively. When deviations from normality and homogeneity were detected a non-parametric test were applied. Parametric

comparisons were performed using the Student t-test.

Comparisons among monthly HSI and GSI were performed using the non-parametric Kruskal-Wallis' H-test (SOKAL & ROHLF, 1995). The variables range was expressed, along with the mean value and the standard deviation, as ' $\text{range (mean} \pm \text{SD)}$ '. The significance level considered in all cases was 0.05.

RESULTS

Description of the male reproductive stages

There were a total of 396 males recorded, ranging from 13.3 to 58.5 cm TL. Immature males ranged from 16.8 to 47.0 cm TL. Clasper length in this stage varied from 0.2 to 5.8 cm (2.19 ± 1.23 , $n = 208$, Fig. 2A) and clasper gland length from 0.7 to 2.8 cm (1.70 ± 0.51 , $n = 60$), with the gland evident for TL of 29.5 cm and longer (Fig. 2B). Testicles weight ranged from 0.1 to 3.0 g (0.89 ± 0.80 , $n = 115$, Fig. 3A) and lobes diameter from 0.1 to 0.7 cm (0.28 ± 0.15 , Fig. 3B) in immature specimens.

Juveniles ranged from 42.5 to 48.0 cm TL. In this stage, clasper length ranged from 6.0 to 12.9 cm (8.38 ± 1.83 , $n = 18$, Fig. 2A) and clasper gland length from 1.8 to 3.2 cm (2.50 ± 0.49 , $n = 11$, Fig. 2B). Testicles varied from 1.0 to 6.0 g (2.46 ± 1.28 , $n = 19$, Fig 3A) in weight

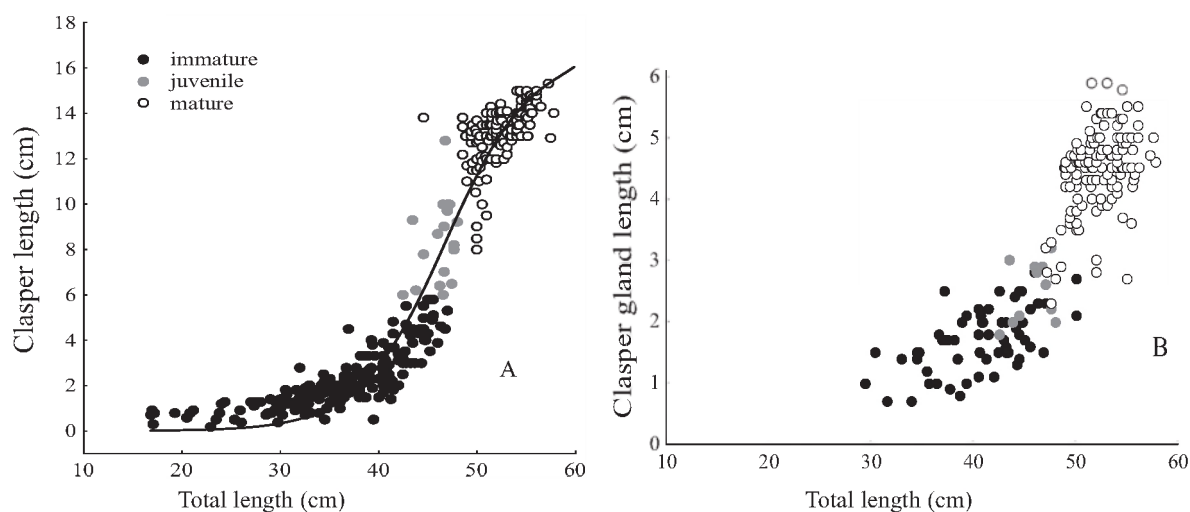


Fig. 2. Relationship between total length and (A) clasper length (along with the logistic curve adjusted) and (B) clasper gland length for immature, juvenile and mature males of *Atlantoraja cyclophora*

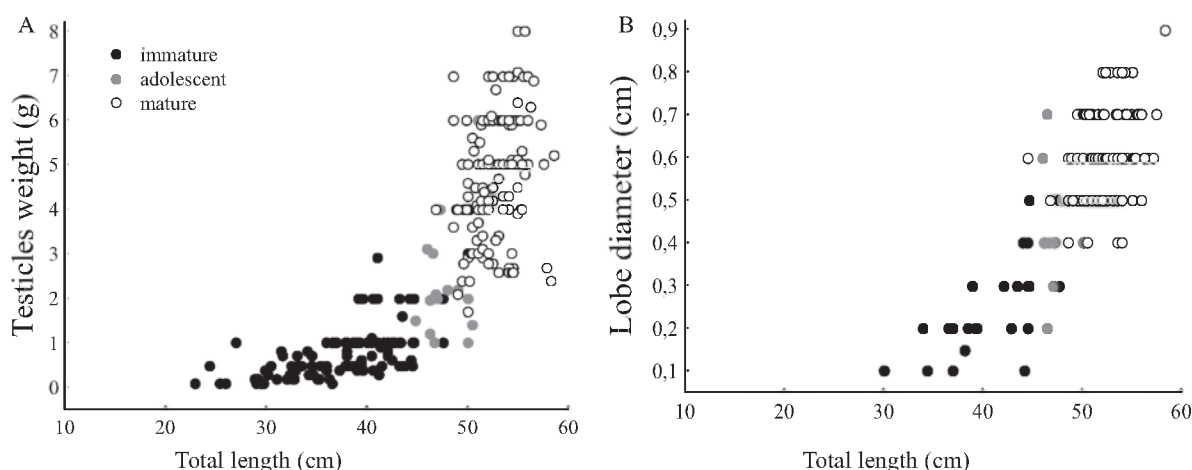


Fig. 3. Relationship between total length (cm) and (A) testicles weight (g) and (B) diameter of the testicles' lobes (cm) for immature, juvenile and mature males of *Atlantoraja cyclophora*

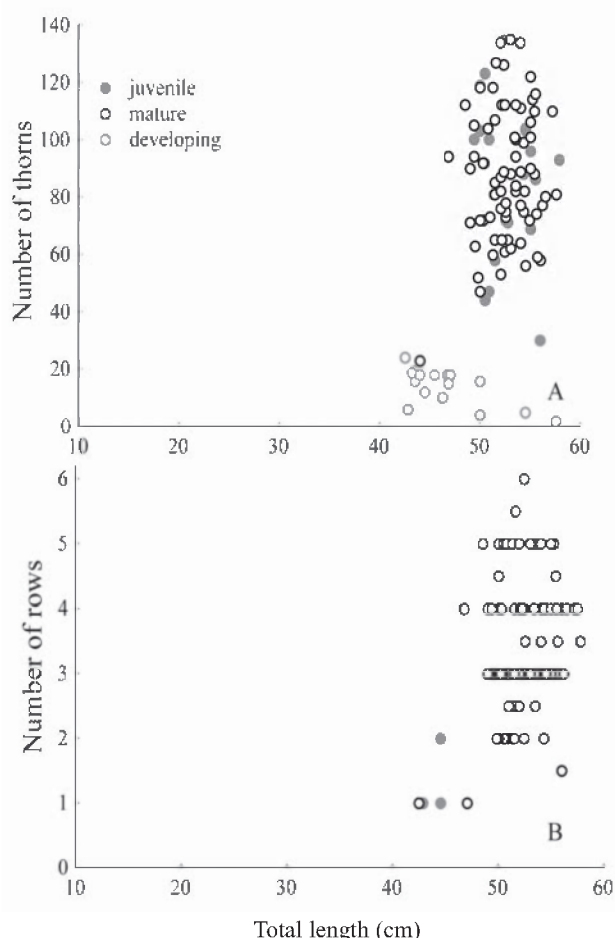


Fig. 4. Relationship between total length (cm) and (A) number of alar thorns and (B) number of rows of alar thorns for juvenile and mature males of *Atlantoraja cyclophora*

and lobes from 0.2 to 0.7 cm in diameter (0.46 ± 0.12 , $n = 15$, Fig. 3B). The number of alar thorns per pectoral fin in juveniles varied from 3 to 71 in the right and 3 to 70 in the left pectoral fin (Fig. 4A). Developing thorns varied from 2 to 12 in the right and 2 to 13 in the left pectoral fin, being mostly present in juvenile males. The number of alar thorns rows varied from 1 to 3 per fin (Fig. 4B). Fully formed alar thorns began to occur at TL of 53.0 cm.

Mature males ranged from 44.5 to 57.8 cm TL, with claspers varying from 8.0 to 15.3 cm in length (13.11 ± 1.33 , $n = 134$, Fig. 2A). The inflexion point of the logistic curve adjusted to the clasper length / TL ratio resulted in 47.0 cm ($R = 0.98$, $a = 10.79$, $b = 0.23$, $n = 208$). Clasper gland growth was continuous throughout development, seemingly describing a potential curve, with length varying from 2.3 to 5.9 cm (4.48 ± 0.67 , $n = 127$, Fig. 2B). Testicles' weight ranged from 1.7 to 8.0 g (4.40 ± 1.43 , $n = 134$) with lobes ranging from 0.6 to 0.9 cm (0.61 ± 0.11 , $n = 120$) (Fig. 3B).

In mature males the number of alar thorns per fin varied from 3 to 71 in the right and 3 to 70 in the left pectoral fin (Fig. 3A) with no significant difference between means (42.5 ± 13.1 , $n = 92$ and 42.9 ± 12.7 , $n = 92$, respectively; $t = -0.2046$, d. f. = 181, $p = 0.7714$). The number of alar thorn rows varied from 1 to 6 in both pectoral fins (Fig. 3B).

Description of the female reproductive stages

A total of 401 females ranging from 11.5 to 68.0 cm TL were analysed. Immature females ranged from 19.5 to 53.0 cm TL. Ovaries' weight varied from 0.1 to 4.3 g (0.97 ± 1.07 , $n = 195$) and uteri width from 0.1 to 1.2 cm (0.35 ± 0.19 , $n = 107$, Figs. 5A and 5B). Nidamental gland width varied from 0.1 (not yet differentiated from the oviduct) up to 1.9 cm (0.60 ± 0.50 , $n = 78$, Fig. 6A). Follicles' diameters varied from 0.1 to 0.5 cm (0.17 ± 0.11 , $n = 36$), being white in appearance (Fig. 6B).

Juvenile females varied in TL from 50.5 to 58.5 cm. Ovaries' weight varied from 2.9 to 11.0 g (5.56 ± 2.77 , $n = 12$) and uteri width from 0.6

to 2.2 cm (1.33 ± 0.47 , $n = 11$, Figs. 5A and 5B). Nidamental gland width ranged from 1.1 to 2.9 cm (2.00 ± 0.44 , $n = 18$, Fig. 6A). Vitellogenesis begins when follicles attain 0.9 cm in diameter, with the follicles being light yellow in colour and with diameters varying from 0.4 to 1.0 cm (0.69 ± 0.22 , $n = 16$, Fig. 6B).

Mature, non egg-bearing females ranged from 56.0 to 68.0 cm in TL. In these females, ovaries ranged from 7.0 to 100 g (28.04 ± 15.40 g, $n = 53$) in weight and uteri from 0.3 to 2.8 cm (1.95 ± 0.50 , $n = 46$) in width. However, with the exception of a female with a 100.0 g ovary, weight varied between 7.0 and 52.8 g (Fig. 5A). In that female, the right ovary was anomalous and bore 10 vitellogenic follicles, with a volume

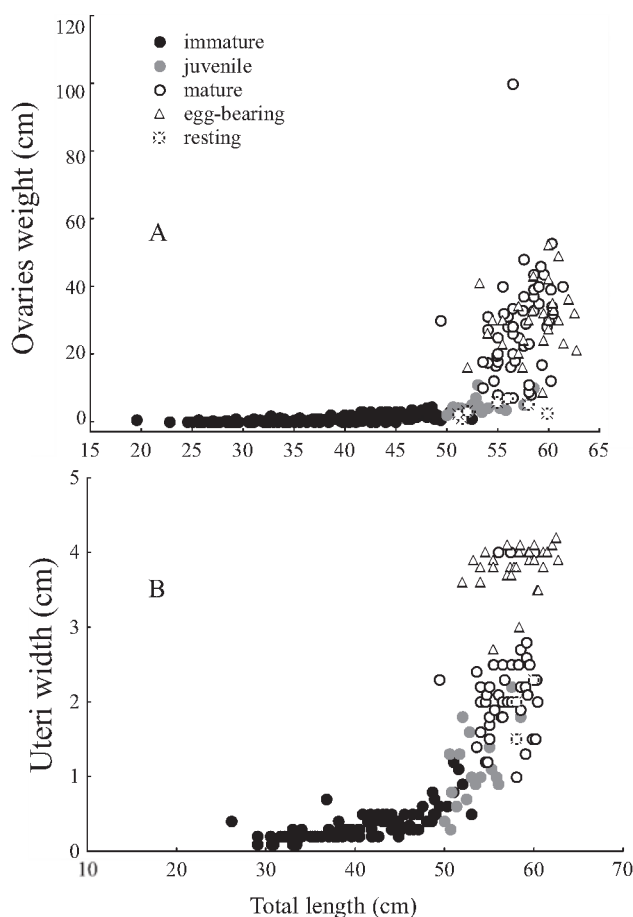


Fig. 5. Relationship between total length (cm) and (A) ovaries weight (g) and (B) uteri width (cm) for immature, juvenile and mature females of *Atlantoraja cyclophora*

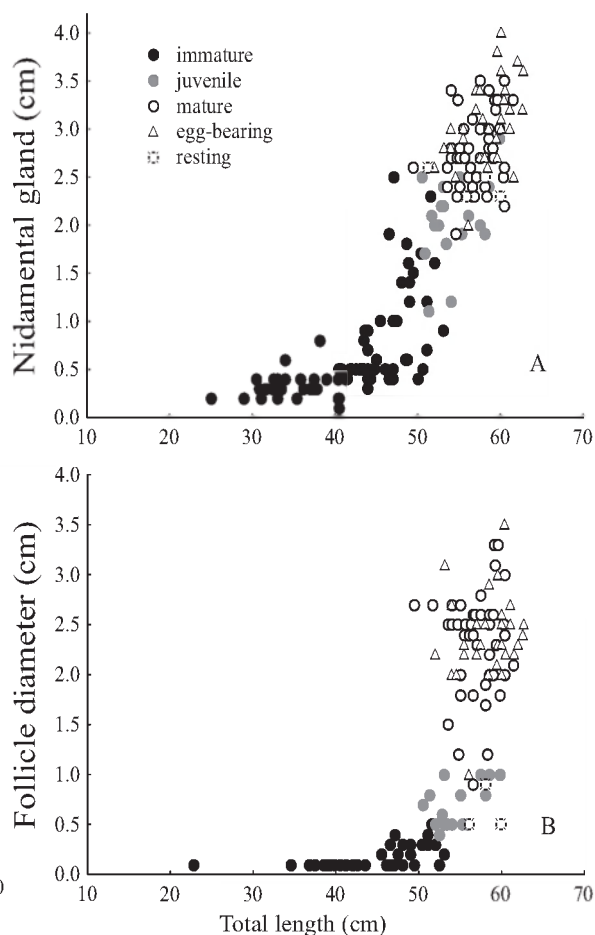


Fig. 6. Relationship between total length (cm) and (A) nidamental gland width (cm) and (B) largest ovarian follicle diameter (cm) for immature, juvenile and mature females of *Atlantoraja cyclophora*

of 9.0 ml and a diameter of 4.5 cm. The typical orange colouration of *A. cyclophora*'s vitellogenic follicles was absent and instead, follicles were light, pale yellow, with an abnormal stiff surface that did not suffer deformation upon exerting pressure and manipulation as in fresh normal ovarian follicles. The left ovary, in contrast, bore vitellogenic follicles of 0.7 cm, most of them damaged preventing the measurement of diameters, and was ~15.0 g in weight.

In egg-bearing females (59.4–62.7 cm TL), ovaries ranged from 8.7 to 52.0 g (30.0 ± 9.80 , $n = 28$) in weight and uteri from 2.7 to 4.2 cm (3.83 ± 0.30 , $n = 35$, Figs. 5A and 5B) in width. Nidamental glands varied in width from 1.9 to 3.5 cm (2.77 ± 0.38 , $n = 47$) in non egg-bearing females and from 2.0 to 4.0 cm (3.10 ± 0.43 , $n = 34$) in egg-bearing females (Fig. 6A). Follicle diameter varied from 1.2 to 3.3 cm in mature females with empty uteri (2.31 ± 0.53 , $n = 42$) and from 1.0 to 3.5 cm in egg-bearing females (2.40 ± 0.44 , $n = 29$, Fig. 6A).

Modal value of the largest ovarian vitellogenic follicle in the adult female was 2.5 cm. The proportion of these follicles was the same in both egg-bearing and non egg-bearing females. As ovulation in egg-bearing females was observed to occur immediately after egg-laying, it was assumed that follicles with diameters ≥ 2.0 cm were pre-ovulatory. The volume of the largest ovarian follicle varied between 3.0–5.0 ml (4.60 ± 0.70 , $n = 9$) and the size of the fertilised ovule inside the egg capsule was 3.0 ml ($n = 3$). According to these observations, the volume of the pre-ovulatory ovarian follicle is 5.0 ml, which corresponds to a follicle diameter of ~3.0 cm.

Females with egg capsules in formation had vitellogenic follicles with diameters between 0.9–2.2 cm ($n = 3$). The number of vitellogenic follicles varied from 2 to 18 (8.90 ± 3.70 , $n = 30$) in non egg-bearing females and from 1 to 18 (mean = 9.70, SD = 3.50, $n = 23$) in egg-bearing females (Fig 7A).

There were six sexual resting females recorded, with TL ranging from 51.2 to 59.9 cm. These females were caught in May, June, July, September and November. Resting ova-

ries weight varied from 2.1 to 6.8 g (4.11 ± 1.92 , $n=6$) and bore white ovarian follicles with diameters between 0.5 and 0.9 cm ($n=3$). Nidamental glands and uteri had adult characteristics (as typical adult dimensions and, in the case of uteri, vascularisation and distension), and ranged from 2.3 to 2.6 cm (2.40 ± 0.15 , $n=4$) and 0.9 to 4.0 cm (2.30 ± 1.28 , $n = 4$) in width respectively. A 58.0 cm TL female classified as mature had a normal, vitellogenic left ovary, with follicles of 1.7 cm in diameter. However, the right ovary presented white follicles with immature characteristics.

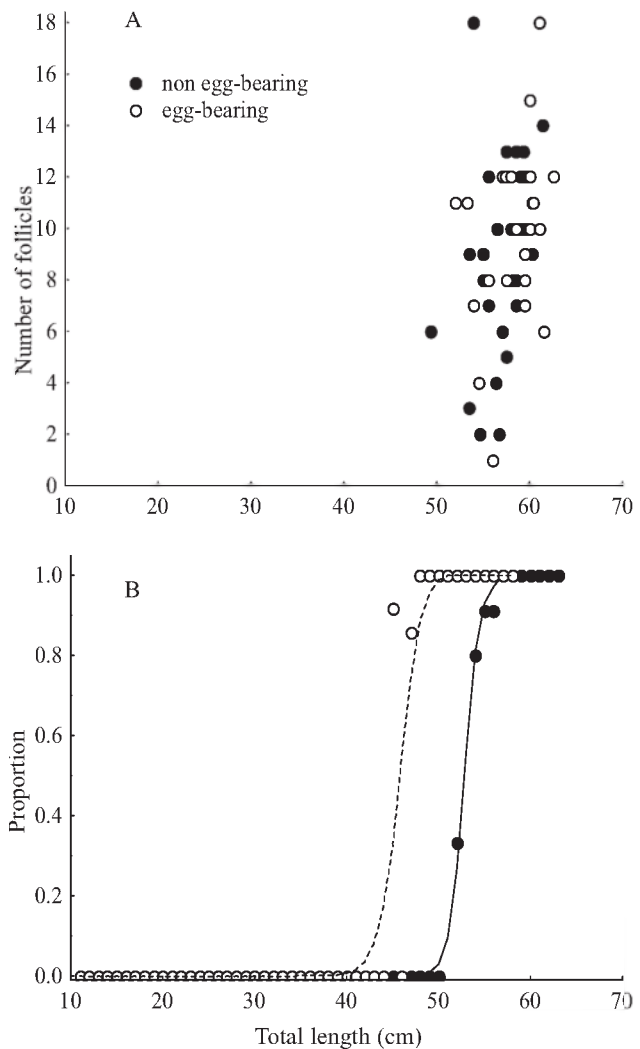


Fig. 7. Relationship between total length and (A) number of vitellogenic follicles and (B) proportion of mature males (empty circles; dotted line) and females (full circles; continuous line) of *Atlantoraja cyclophora*

Estimation of size-at-maturity

Both the smallest mature male and the largest immature male were 47 cm long. Males' TL_{50} was calculated to be 46.3 cm ($R=0.99$, $a=39.83$, $b=0.86$, $n=49$), which corresponded to 78.3% of the maximum TL observed for the males of this species in this area. The smallest mature female was 51.6 cm long while the largest immature female was 53.0 cm long. Size-at-maturity was calculated to be 53.2 cm ($R=0.96$, $a=65.87$, $b=1.24$, $n=52$) for the females, which corresponded to 84.0% of the maximum TL observed (Fig. 7B).

Reproductive cycle

For the males, HSI varied between 0.48 (August) and 3.54 (November) without significant variation for the fourteen-month period ($H_{(12, 169)}=0$, $p=1.0000$) (Fig. 8A). The GSI varied between 0.15 (November) and 1.45 (June), also with significant differences among months ($H_{(12, 156)}=0$, $p=1.0000$) (Fig. 8B).

For the females HSI varied from 1.55 and 6.30 (both for April 2006) without significant variation for the fourteen-month period ($H_{(12, 86)}=21$, $p=0.0504$) (Fig. 8A). The GSI varied between 0.08 (December) to 4.41 (October), also with significant differences among months ($H_{(12, 78)}=0$, $p=1.0000$) (Fig. 8B).

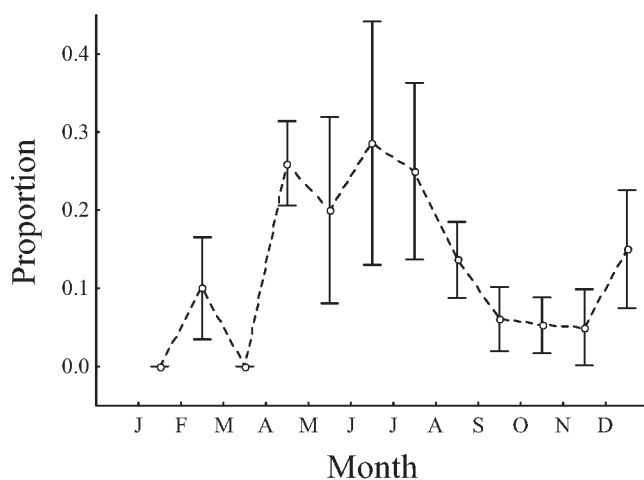


Fig. 9. Monthly variation of the proportion of egg-bearing females of *Atlantoraja cyclophora* on an annual basis. Whiskers represent the standard deviation of the proportion

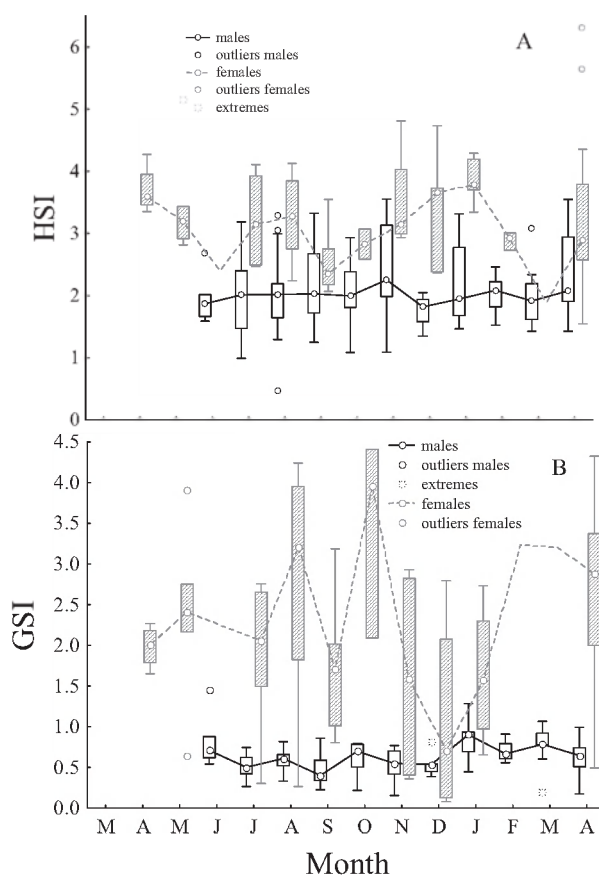


Fig. 8. Seasonal variation of (A) hepatosomatic (HSI) and (B) gonadosomatic (GSI) indices for males and females of *Atlantoraja cyclophora*, from March 2005 to April 2006. Whiskers represent the non outlier range. The box between the lower (25%) and upper (75%) quartiles represent the middle 50% of the HSI and GSI respectively, and middle point represents the median value

Egg-bearing females occurred in all months with proportions varying from 0.030 (March) to 0.67 (April) (Fig. 9). According to the results, the proportion of egg-bearing females would peak between April and July when values of 0.20 to 0.29 would be attained.

DISCUSSION

Sexual development of males and females

Immature, juvenile and adults of *A. cyclophora* did not exhibit a significant overlap in their size ranges. Well-delimited maturity stages with regard to TL were also noted in *A. castelnaui* and *A. platana* (ODDONE & AMORIM, 2008; ODDONE 2008, personal communication). Conversely, in species like *Rioraja agassizi*, a high overlap among maturity stages occurring in both sexes was noted, especially regarding testicles and the number of alar thorns in males, and ovary weight and the number of follicles in females.

Developing thorns are also common in juvenile and adult stages of *A. cyclophora* and *R. agassizi* (ODDONE & VOOREN, 2005; ODDONE *et al.*, 2007). ODDONE & VOOREN *op. cit.* also recorded up to 6 rows of alar thorns in *A. cyclophora*. In *A. platana* ODDONE & AMORIM (2008) noted a maximum number of 5 rows and in *R. agassizi* up to 5 (ODDONE *et al.*, 2007). On the other hand, *A. castelnaui*, the largest species of the genus, has a lesser number of rows of up to 3 per pectoral fin (ODDONE *et al.*, 2008). The number of alar thorns rows may be therefore be species-specific.

The plot of the clasper length/TL ratio was a typical three-phased sigmoid curve observed in rajid species (e.g. CAPAPÉ, 1974; CAPAPÉ & QUIGNARD, 1974; TEMPLEMAN, 1987; ODDONE & VELASCO, 2004; ODDONE *et al.*, 2007). Such a pattern, however, was not observed in *Atlantoraja castelnaui* and *A. platana*, where continuous clasper growth in the mature phase was noted (ODDONE, 2008 personal observation). A similar pattern in the siphon gland/TL ratio was recorded for *Rioraja agassizi* (ODDONE *et al.*, 2007).

ODDONE & VOOREN (2005) noted that vitellogenesis began when follicles attained a diameter of 0.9 cm in *A. cyclophora*. In *A. platana* vitellogenesis was observed to start when follicles attain 0.7 cm in diameter; in *A. castelnaui* at 1.0 cm and in *R. agassizi* at 0.5-0.6 cm diameter (PONZ LOURO 1995; ODDONE *et al.*, 2007; ODDONE, 2008 personal observation). These observations indicate that follicular size at the onset of vitellogenesis is proportional to the species maximum size and consequently, strongly species-specific.

According to the analysis of the largest vitellogenic follicle in egg-bearing females and mature females of *A. cyclophora* with empty uteri, follicles attain ovulatory size upon attaining a diameter of ~3.0 cm. For this species this size had been previously estimated at 2.6 cm diameter in southern Brazil (ODDONE & VOOREN 2005). In *A. castelnaui* ovulatory size is 3.0 cm, whilst in *R. agassizi* it is 2.0 cm in diameter (ODDONE *et al.*, 2007; ODDONE 2008, personal communication).

A maximum number of 18 ovarian vitellogenic follicles were recorded in this study for *A. cyclophora*. For *A. platana*, up to 12 (in an egg-bearing female), and in *A. castelnaui* up to 20 (also in an egg-bearing female), follicles were observed whereas in *Rioraja agassizi* up to 30 were noted (ODDONE *et al.*, 2007; ODDONE, 2008 personal observation). LICANDEO *et al.* (2007) recorded up to 62 follicles in *Dipturus chilensis* and LICANDEO *et al.* (2007) found a maximum number of 68 follicles in *D. trachyderma*. EBERT (2005) noted that for six of eight *Bathyraja* species examined, the total number of mature follicles increased with TL, with the number of follicles for one species (*B. aleutica*) increasing up to a given size (145 cm TL) and then declining in larger individuals.

Size-at-maturity

As previously reported by ODDONE & VOOREN (2005) for southern Brazil, females of *A. cyclophora* attained maturity at a larger size than males, as also noted for the remaining *Atlantoraja* species (ODDONE, 2008 personal observation)

and for several other skate species. EBERT (2005), however, noted that for seven of eight *Bathyrāja* species, both females and males matured at approximately the same TL, with only one species (*B. aleutica*) where females matured at a much larger size than males. For the species in question, ODDONE & VOOREN (op. cit.) calculated LT_{50} to be 48.5 (males) and 52.8 cm (females). Comparing with this study, these values are close to that found for the females (53.2 cm) and for the males ($LT_{50}=46.3$).

LICANDEO & CERNA (2007), for *Dipturus chilensis* from two locations in Chilean Pacific waters (from the southern fjords and the fjords of Chilean Patagonia), also did not record differences in the LT_{50} between regions. This pattern contrasts with reports on geographic variations in the size-at-maturity in skates, especially with regard to latitudinal position, e.g. TEMPLEMAN (1987) and MABRAGAÑA & COUSSEAU (2004). ODDONE *et al.* (2007) recorded values of LT_{50} for *Rioraja agassizi* in southeastern Brazil considerably lower than COLONELLO *et al.* (2007) for the same species in Argentinean waters (see Introduction section). According to LICANDEO & CERNA (2007) a link between an increase in latitude (or a decrease in temperature) and an increase in body size and size at maturity, though presently poorly understood, would be expected.

The estimates of LT_{50} were smaller than the size of the smallest mature individual observed (47.0 cm for males and 51.6 cm for females). EBERT (2005) also noted this fact in several *Bathyrāja* species, arguing that this could be related to factors such as a small sample size and the consequent lack of, or few replicates, of individuals within the same size class. However, in this work, sample size was large (396 and 401 male and female specimens respectively). In addition, BRACCINI & CHIARAMONTE (2002), also working with a large sample size, noted that in *Psammobatis extenta* the onset of maturity occurred at only 1 and 1.3 cm, for males and females, respectively, below the estimated LT_{50} . ODDONE, 2008 personal observation, also reported LT_{50} values lower than observed maturity values for *Dipturus chilensis*, suggesting to bear

in mind that the size at maturity is an observed value and LT_{50} is a theoretical one.

Males were found to mature at 78.3% and females 84.0% of their maximum TL observed. ODDONE & VOOREN (2005) found values of 76% and 82% respectively for this species. Males of *A. platana* mature when they have attained 89%, and females 94%, of their maximum TL observed while in *A. castelnaui* these values are 91% and 83% respectively (ODDONE, 2008 personal observation). In *Rioraja agassizi*, both sexes mature at 68% of the maximum size (ODDONE *et al.*, 2007). Late maturity could therefore be a pattern of the genus *Atlantoraja*. EBERT (2005) noted that in species of genus *Bathyrāja* first maturity occurred at 80% of the maximum TL.

Reproductive cycle

Skates are serial spawners and are able to deposit egg capsules even with daily frequency during the peak egg-laying time (CLARK, 1922; HOLDEN *et al.*, 1971; ELLIS & SHACKLEY, 1995). Egg-laying in *A. cyclophora* was noted all year round, with higher proportions of egg-bearing females from April to July. *Raja pulchra* undergoes egg-laying throughout the year except in August and September, peaking from April to June and November-December (YEON *et al.*, 1997). In *Rioraja agassizi* egg-laying occurs continuously during the year peaking twice, in September and December (ODDONE *et al.*, 2007). CAPAPÉ *et al.* (2007) observed that in *Raja clavata* (northern Mediterranean Sea), vitellogenesis occurred throughout the entire year, with a diminution in April and August just when the production of egg capsules was not observed.

In the southwest Atlantic, *Psammobatis extenta* bore egg capsules throughout the year (BRACCINI & CHIARAMONTE, 2002) but the maximum proportion of egg-bearing females occurred during summer, whereas *Sympterygia bonapartii* seem to carry egg cases only in summer (MABRAGAÑA *et al.*, 2002). WALKER (1999) noted that in *Raja naevus*, egg-bearing females only occur from July to September, when the highest proportion of egg-bearing females of *R. radiata* also was noted.

LICANDEO & CERNA (2007) recorded female *Dipturus chilensis* carrying egg capsules throughout the year for two localities (southern and Patagonian fjords); with an increase in the proportion of post-partum females and a decrease in their GSI during summer, this pattern is consistent with trends exhibited by males. Therefore, female *A. cyclophora* undergoes an annual cycle, with slight seasonal variations in the reproductive activity (not statistically significant). In *Rioraja agassizi* the cycle is annual with ovulation and egg-laying occurring throughout the year, with lowest values of GSI in spring and summer and maximum values in the HSI in summer-autumn for males and females (ODDONE *et al.*, 2007).

ODDONE & VOOREN (2005) proposed for *A. cyclophora* in southern Brazil (working with a two-season sample set), either an annual cycle with continued reproductive activity and no peaks or an annual cycle with at least one peak in reproductive activity in spring and/or autumn. In the present paper, working with monthly samples for a whole year, it was demonstrated that the second hypothesis better fits the results. Annual reproductive cycles have been largely documented in the literature for rajids. Species like *Raja clavata* and *Dipturus chilensis* are known to undergo annual reproductive cycles (HOLDEN, 1975; FUENTEALBA & LEIBLE 1990). LICANDEO & CERNA (2007) noted a peak in the reproductive activity of *D. chilensis* in Chilean waters. The reproductive activity in males of *A. cyclophora* presumably takes place continuously during the year with much less variation in the HSI and GSI than in the females.

Sexual resting females were recorded in several rajid species (*e.g.*, HOLDEN *et al.* 1971; CAPAPÉ 1974, 1976; ODDONE & VOOREN 2005). EBERT (2005) noted that in two of the largest females of *Bathyraja aleutica*, though meeting the adult criteria, had ovaries that appeared to have ‘atrophied’ and that individuals of two additional species (*B. lindbergi* and *B. minispinosa*) were found to be reproductively inactive as their ovaries appeared to have ‘atrophied’. This author argued that the occurrence of

those females suggests either that a period of diapause or reproductive inactivity within the population occurs or that these individuals had reached the end of their reproductive viability and had senesced.

This question remains unanswered and needs further study. However, it is important to note that an adult ovary of reduced size and with transparent colourless follicles (*i.e.* ovarian follicles in the initial stage of development) is in the “resting stage” and is not necessarily “atrophied”, as the last term denotes a pathological situation or, in the case of the ovary, perhaps the state of the ovary in the senescent female that no longer breeds. The 58.0 cm TL recorded in the present work was presumably either a female about to undergo the sexual resting period or restarting folliculogenesis and vitellogenesis after it.

CONCLUSIONS

Even when collected as by-catch, skates are often subjected to high fishing mortality and, as a consequence, some species (not only of skates but also other elasmobranchs) have been extirpated from large regions (STEVENS *et al.*, 2000). At least nine skate species have already disappeared from their distribution ranges (BRANDER, 1981; DULVY & REYNOLDS, 2002). Because of the different growth and maturation characteristics among rajids, species will be affected differently by size-selective mortality imposed by fishing activity (WALKER, 1999). So far, *A. cyclophora* has been intensely exploited as a by-catch and marketed, yet is already considered by the IUCN Red List of Threatened Species as ‘vulnerable’, as mentioned previously. For the congenerical species, the status of *A. platana* remains unknown although *A. castelnaui* has become an ‘endangered’ species (HOZBOR *et al.*, 2004).

Knowledge of the reproductive biology, and namely the cyclicity of the reproduction, together with growth parameters and fishing mortality estimations will be crucial for the development of management plans for protecting the populations of the genus *Atlantoraja*

from commercial extinction and disappearance. So far, in skates, the impacts of fishing activity upon mating, egg-laying, nursery grounds and mortality of egg capsules and juveniles, is not known (WALKER, 1999).

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Seksualni razvoj i reproduktivni ciklus raže okatice *Atlantoraja cyclophora* (Regan, 1903), (Chondrichthyes: Rajidae: Arhynchobatinae) u južnom Brazilu

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SAŽETAK

Jedinke raže okatice, *Atlantoraja cyclophora* prikupljene su mjesečno iz gospodarskih lovina u Guarujá-u, država São Paulo, Brazil, od ožujka 2005. do travnja 2006. na dubinama od 10 do 146 metara. Duljina mužjaka kolebala je od 13.3 do 58.5 cm TL (n=396). Najmanji spolno zreli mužjak i najveća spolno nezrela ženka bili su dugi 47.0 centimetara. Izračunata dužina mužjaka pri stadiju 50% zrelosti populacije je iznosila 46.3 cm. Dužina ženki se kretala u rasponu od 11.5 do 68.0 cm (n=401). Najmanja spolno zrela i najveća nezrela ženka su bile dugačke 51.6 cm, odnosno 53.0 cm. Izračunata dužina ženki pri stadiju 50% zrelosti populacije je iznosila 53.2 cm. Kod mužjaka su hepatosomatski i gonadosomatski indeks kolebali od 0.48 (kolovoz) i 3.54 (studeni) i između 0.15 (studeni) i 1.45 (lipanj) bez značajnih kolebanja tijekom razdoblja od četrnaest mjeseci. Kod ženki su hepatosomatski i gonadosomatski indeks kolebali od 1.55 do 3.54 (oba u travnju 2006.) i između 0.08 (prosinac) i 4.41 (listopad), bez značajnijih mjesečnih kolebanja. Ženke s jajima su bile nazočne tijekom svih mjeseci u razmjerima koji su kolebali od 0.03 (ožujak) do 0.67 (travanj). I mužjaci i ženke prolaze kroz svoj godišnji ciklus, sa neznatnim sezonskim kolebanjima u reproduktivnoj aktivnosti i maksimumom u proporciji ženki s jajima između travnja i srpnja.

Ključne riječi: kopulatorni organ, hrskavičnjače, folikule, nošenje jaja, gonade, reprodukcija, spolno neaktivno razdoblje