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Age, Growth, Mortality, Longevity and Reproductive Biology of the White Skate, *Rostroraja alba* (Chondrichthyes: Rajidae) of the Gulf of Gabès (Southern Tunisia, Central Mediterranean)

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Abstract

The Age, growth, mortality, longevity and reproductive parameters were studied for *Rostroraja alba* from the Gulf of Gabès (Southern Tunisia, central Mediterranean Sea), collected monthly during 2007 from commercial fisheries. 43.75% of the composition was females and 56.25% males. A total of 140 females (44.5-160 cm TL) and 180 males (39.5-150 cm TL) were examined to study the reproductive cycle. A subsample of 115 specimens (65 females and 50 males, ranging from 39.5 to 160 cm TL) was used for age and growth estimation derived from vertebral centra.

The oldest female was 35 years and 160 cm TL, whereas the oldest male was 32 years and 150 cm TL. The von Bertalanffy growth parameters were L_∞= 177.6±3.61 cm, k= 0.06±3.08 year^{-1} and t_0= -1.28±1.97 10^{-1} year for females and L_∞= 199.6±3.66 cm, k= 0.04±1.9610^{-3} year^{-1} and t_0= -1.47±0.35 10^{-1} year for males. Growth was not significantly different between sexes.

The maturity size (TL 50%) was 119.3 cm (94.20 cm DW) for males and 129.4 cm (95.77 cm DW) for females. The maturity age (A50%) was estimated to 19.69 and 23.47 years respectively for males and females. Size and age maturity were not significantly different between sexes.

Keywords: *Rostroraja alba*, age, growth, reproduction, Gulf of Gabès, Mediterranean Sea

Introduction

The white skate, *Rostroraja alba* is distributed in the Eastern Atlantic from the British Isles southward, also along the coast of Africa and in most of the Mediterranean (to Tunisia and Turkey) (Dulvy *et al.*, 2006; Froese and Pauly, 2006; Fricke *et al.*, 2007). The IUCN 2012 Red List for chondrichthyans in the Mediterranean Sea assessed the status of *R. alba* as "Endangered" (Abdul Malak *et al.*, 2011).

Although this species is rarely recorded in the Mediterranean coasts (Serena, 2010), of this reason, there is a paucity of information about biology and distribution of the white skate.

These recommendations aim to encourage providing the data needed to develop appropriate conservation and in the Mediterranean Sea, no studies on age growth, longevity and mortality of *R.alba* were reported, but very little information about its reproductive biology has been examined in some restricted areas (Capapé, 1974). In fact, off the Tunisian coasts, *R. alba* is captured essentially in the spring and summer. It is taken mainly as by-catch of bottom trawl fisheries, in the Gulf of Gabès, although there are researchs on the age and growth of some elasmobranch in the Mediterranean Sea (Duman and Başusta, 2013; Enajjar *et al.*, 2012; Kadri *et al.*, 2012; Başusta and Sulikowski, 2012; Yigin and Ismen, 2010; Yeldan *et al.*, 2009; Başusta *et al.*, 2008), this is the first study on the life history of *R. alba* in the Gulf of Gabès.

Age information is crucial to our understanding of the resilience of species to exploitation because it forms the foundation for calculations of growth and mortality rates, age at maturity and longevity (Campana, 2001).

The purpose of the current study is to: (1) provide biological information about age growth, longevity and natural mortality of this species (2) estimate size and age at sexual maturity for both sexes. (3) characterise the reproductive cycle based on morphological changes in reproductive organs for both sexes.

Materials and Methods

Samples of *R. alba* were collected in this study from commercial bottom trawlers landings in the Gulf of Gabès between January and December 2007. The commercial trawlers use a 22 mm stretched mesh size cod-end and operate over 30 m depth (Figure 1).

For each specimen, the total length (TL) and the disc width (DW) were recorded to the nearest centimeter. For males, the claspers length (LC) was measured as the distance from its tip to the pelvic girdle.

The total mass (TM) of each specimen was weighed to the nearest 10 g. The TL-TM and TL-DW relationships were calculated separately for each sex.

The slopes of the logarithmic transformed relationships TL-TM and TL-DW among sexes were tested by t-tests (Zar, 1996). A block of 10 vertebral centra were taken from above the abdominal cavity of 115 rough skate, vertebrae were cleaned of extraneous tissue with a scalpel, neural, and haemal arches were removed and individual centra were separated.

The vertebrae were soaked in a 5% sodium hypochlorite solution until the material could be removed effectively, then subsequently washed in running water and air-dried (Başusta and Sulikowski, 2012).

One vertebra from each specimen was cut through the focus with a double bladed low-speed saw to create sagittal thin section, which was mounted on glass slides and dry sandpapered to produce a thin section of 0.6 mm (Duman and Başusta, 2013).

Vertebral sections were digitally photographed with a camera attached to an Olympus S2X9 stereomicroscope with a reflected light at ×20 magnification. Growth bands were counted using the image analysis software TNPC 4.1. (Noesis, 2002). Liquid clarifier (EDTA) was applied to the section surfaces to enhance the banding pattern. An annual growth ring was defined as a pair of bands, consisting of one highly calcified (light) band and one less-calcified (dark) band (Wilson *et al.,* 1983).

The first band near the focus was defined as hatch mark (from age zero) (Joung *et al.,* 2011).

Growth band pairs (comprising translucent and opaque bands, interpreted under conditions of reflected light) were counted by four readers for each specimen without prior knowledge of the sex or length of the specimens. The index of the average percentage error (IAPE) (Beamish & Fournier, 1981), the coefficient of variation (CV) (Chang, 1982), and the age-bias plot (Campana *et al.,* 2005), were then used to compare reproducibility of the age determination between readings, as follows:

$$\mathrm{IAPE} = \frac{1}{N}\sum_{j=1}^{N}\left(\frac{1}{R}\sum_{i+1}^{R}\frac{|Xij - Xj|}{Xj}\right)\times 100$$

where *N* is the number of animals aged, *R* is the number of readings, *Xij* is the count from the *jth* animal at the *ith* reading and *Xj* is the mean age of the *jth* animal from i readings.

$$\mathrm{CV} = 100\ \% \times \frac{\sqrt{\sum_{i=1}^{R}(Xij - Xj)^2 \Big/ (R-1)}}{Xj}$$

Where CV is the age precision estimate for the *jth* fish; *Xij* is the age determination of the *jth* fish by the *ith* reader; *Xj* is the mean age of the *jth* fish and *R* is the number of readings.

Vertebral radius (RV) was measured from the focus along the axis of the corpus calcarium to the edge of the vertebra. The relationship between RV and TL was determined.

The periodicity of band pair formation was investigated using both marginal increment ratio

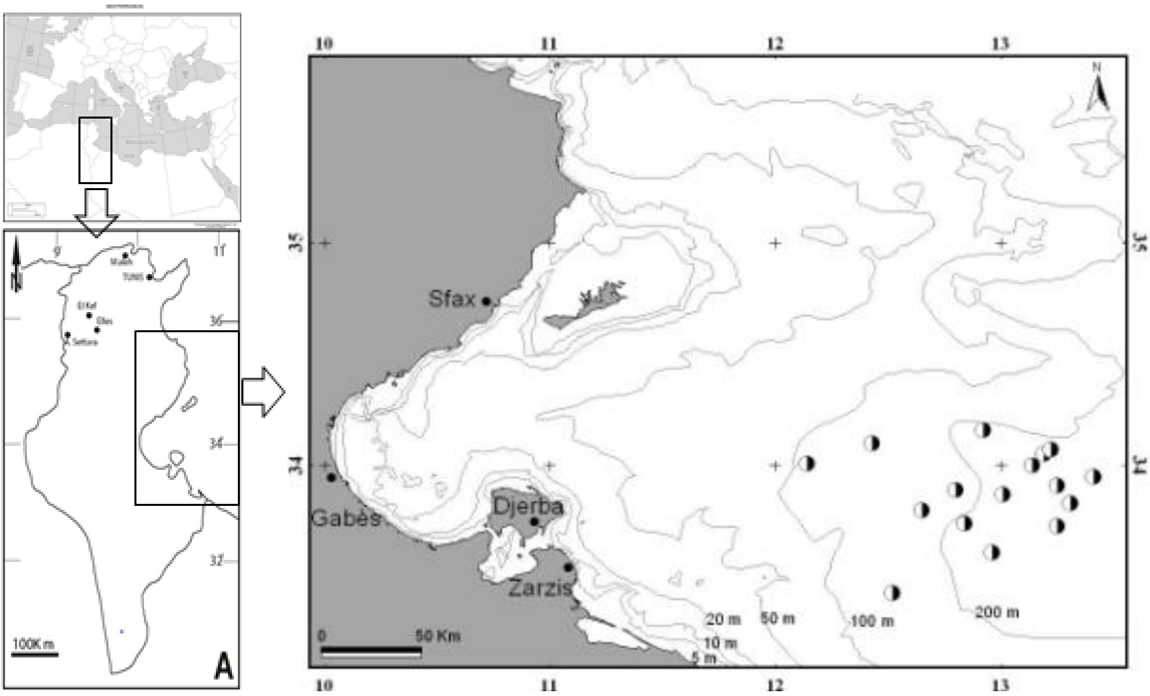

Figure 1. Map of the Gulf of Gabès (Tunisia, Central Mediterranean Sea) and fishing points.

(MIR) (Hayashi, 1976) and edge analysis (Cailliet and Goldman, 2004).

$$MIR = \frac{R - R_n}{R_n - R_{n-1}}$$

Where *R* is the centrum radius, *Rn* and R*n*−1 are radius of the ultimate and penultimate annuli, respectively. Kruskal-Wallis one-way analysis of variance on ranks was used to test for differences in marginal increment by month (Simpfendorfer *et al.*, 2000).

The von Bertalanffy growth equation (VBGE) (von Bertalanffy, 1938) was fitted to the data:

$$TL = TL_{\infty} (1 - e^{-K(t - t_0)})$$

Where TL = total length at age t, TL_{∞} = theoretical asymptotic length, k = growth rate coefficient, and t_0 = the theoretical age at zero length.

The VBGE was calculated by using FISHPARM, a computer program for parameter estimation of nonlinear models with Marquardt's (1963) algorithm for least-square estimation of nonlinear parameters. An analysis of covariance (ANCOVA) was used to compare the slopes between sexes.

The oldest fish aged from the maximum number of external band counts provides an initial estimate of longevity (Irvine *et al.*, 2006) as well as the algorithm A99=5×Ln (2)/k (Fabens, 1965), where A99 is the time (in years) passed before reaching 99% of L_{∞} and k is the growth rate coefficient derived from the Von Bertalanffy growth equation.

The natural mortality rate *M* was estimated using several methods based on maximum age and age at maturity.

$\ln(M)=1.46 - 1.01 \ln(t_{max})$ (Hoenig, 1983)
$M=1.65/ t_{mat}$ (Jensen, 1996)

Where t_{max} is the maximum age in the population and t_{mat} is the median age at sexual maturity.

The reproductive tract of each specimen was removed and examined. The testes and ovaries (with the epigonal organ) mass (MG) and livers mass (MH) were recorded to the nearest 0.01 g. In males, the rigidity and claspers length (LC) were recorded. For each female the uterus width (WU), oviducal gland width (WO); the number, colour and diameter to nearest 0.01 mm of ovarian follicles and the presence of egg capsule in oviducts were recorded. T-test was used to test the symmetry between right and left reproductive organs in mature individuals.

Males and females were sorted into immature, maturing and mature by macroscopic inspection of the reproductive organs according to Walmsley-Hart *et al.*, 1999; Ebert, 2005). In order to relate body size and sexual development, reproductive variables (CL, GM, WU and WO) were analysed in relation to TL.

Seasonal variability of reproductive status in mature males and females was assigned using the gonadosomatic index (IG) and the hepatosomatic index (IH). These indexes were calculated as IG = 100 MG GM^{-1} and IH = 100 HM GM^{-1}.

Monthly differences in mean IG and IH were tested using analysis of variance (ANOVA, P< 0.05) followed by a Tukey's *post-hoc* (Zar, 1996).

The first maturity is defined as the length or age at which 50% of the individuals of each sex were mature. A logistic ogive was fitted to the data using a maximum-likelihood approach in order to estimate the size (TL_{50}) and age (A_{50}) at which 50% of individuals were sexually mature (Roa *et al.*, 1999). An analysis of covariance (ANCOVA) was used to compare size and age at maturity between sexes.

Results

In total, 320 *R. alba* specimens were measured in this study. Females (n= 140) from 44.5 to 160 cm TL (all means presented ± S.E., 79.57±25. 60 cm) and 820-55.200 g TM (8503.67±12074.46 g, n=140), whereas males (n=180) ranged from 39.5 to 150 cm TL (76.16±21.06 cm, n=180) and 620-42.500 g TM (6903.15±8655.78 g, n=140).

Image of longitudinal cross-section of vertebral centrum was presented in Figure 2.

There was no significant difference in the regression slopes between sexes for the TL and DW relationship (t-test=2.25; d.f=320; P>0.05) (Figure 3). Linear regression of TL on WD resulted in the following equation for both sexes: WD=0.75+1.236 TL (n=320, r^2=0.97).

Females and males had similar length-mass relationships, but females reached almost twice the mass of males. Non-linear regression of TM on TL was significantly different between sexes (t-test = 3.17; d.f =320; P<0.05), this relationship of TL-MT is presented separately for each sex: $TM=0.008\ TL^{3.61}$ (n=140, r^2=0.96) for females and $TM=0.0011\ TL^{3.53}$ (n=180, r^2=0.95) for males (Figure 4).

The remaining 115 vertebrae, 65 females (44.5-160 cm TL) and 50 males (39.5–150 cm TL) were used for age estimation. After separate readings, exact agreement of ring counts were reached on 11% differed by one ring, 7% by two rings and 3% by more than three.

An analysis of covariance of TL and RV suggested that the relationship between sexes was not significantly different (ANCOVA, P>0.05).

Vertebrae were determined to be a useful ageing structure based on the positive linear relationship between their size and TL (TL = 237.67RV+5.08, r^2 = 0.93) and (TL = 221.25RV+7.43, r^2 = 0.92) for females and males respectively (Figure 5).

The results of edge analysis and MIR of vertebral thin sections support annual band pair formation (Figure 6). Centrum edge analysis was

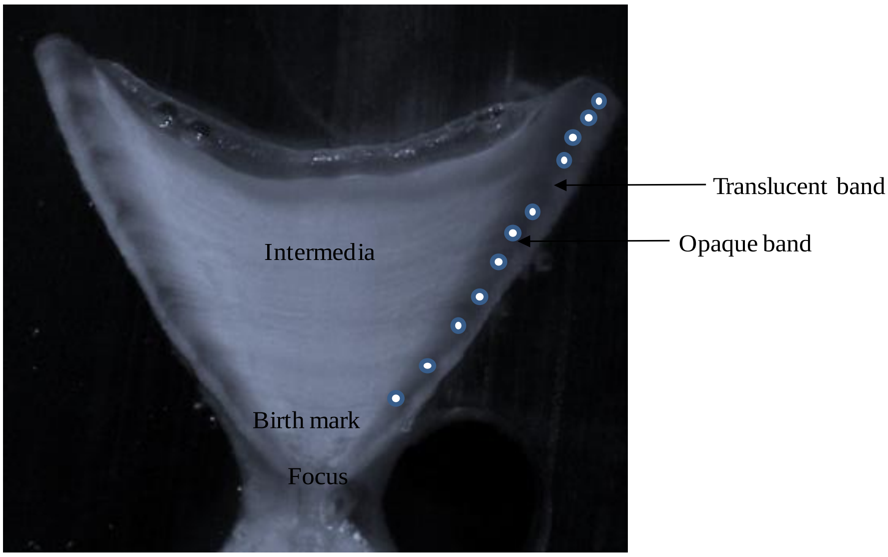

Figure 2. Longitudinal cross-section of vertebral centrum from a 105.5 cm TL female *Rostroraja alba* in the Gulf of Gabès.

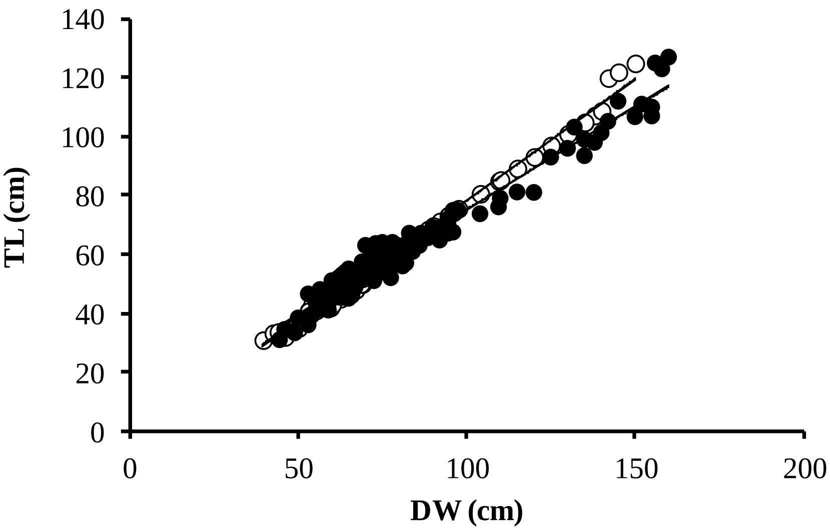

Figure 3. Total length-Width disc relationships for females (●) and males (○) of *Rostroraja alba* in the Gulf of Gabes.

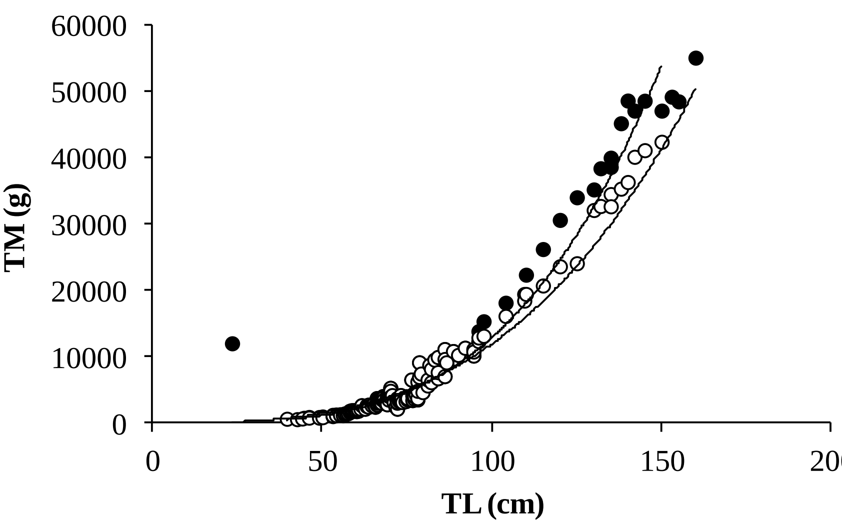

Figure 4. Total length-weight relationships of for females (●) and males (○) of *Rostroraja alba* in the Gulf of Gabes.

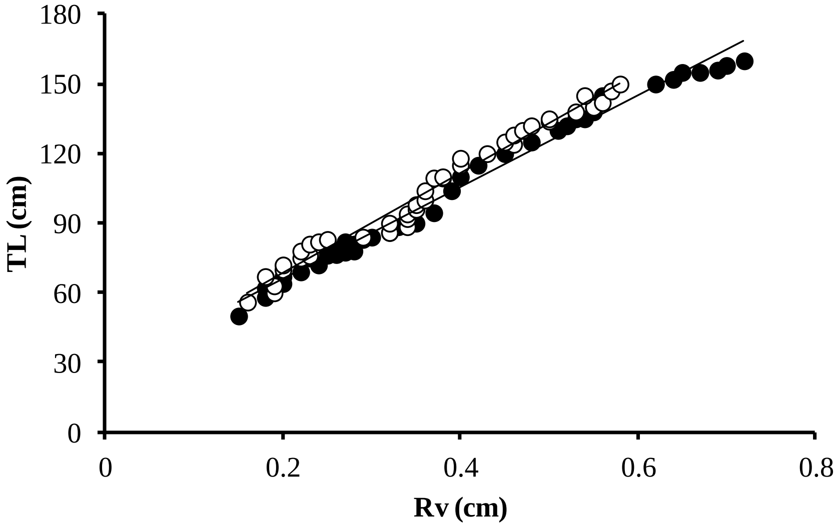

Figure 5. Relationship between total length and vertebral radius of *Rostroraja alba* for females (●) and males (○).

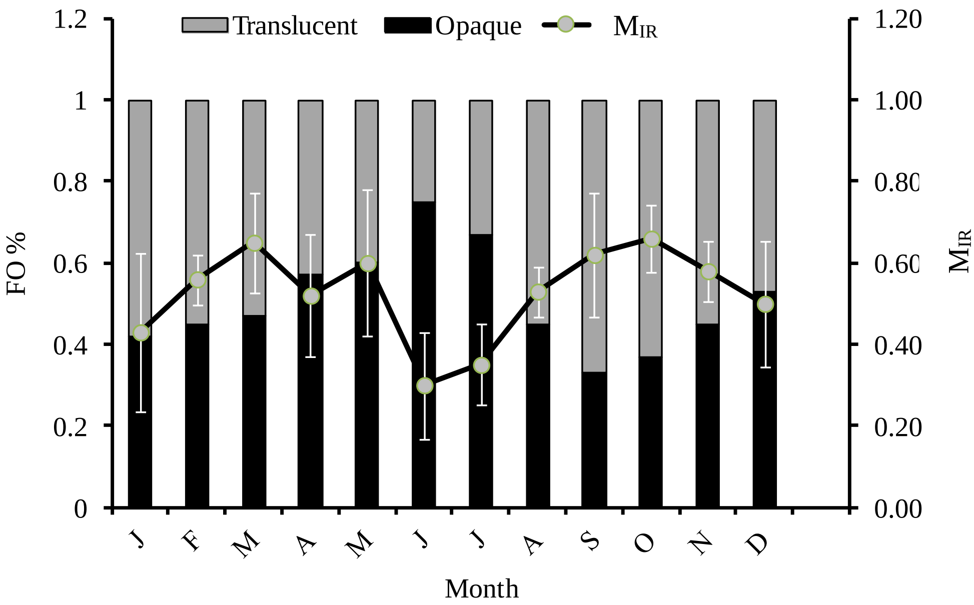

Figure 6. Monthly changes in the mean ± SE. marginal increment (***M_{IR}***) for *Rostroraja alba* (combined sexes).

performed on 115 *R. alba*. The translucent band appeared from September to October while the opaque band appeared between June to July (Figure 6). A significant difference in MIR between months was detected (Kruskal-Wallis, (H = 42.32, dl =11, P <0.05). The lowest mean M_{IR} occurred during June (mean M_{IR} = 0.30±0.02SE), after which an increasing trend was apparent, resulting in the highest mean M_{IR} during the month of October (mean M_{IR}= .66±0.01SE) (Figure 6).

Monthly centrum edge characteristics suggest that a single band pair, comprised of one translucent and one opaque band, is formed within vertebral centra each year.

The average IAPE of the overall sample was 3.15% and the CV was 5.45 for the whole sample. The levels of precision indicated a high level of reproducibility, so these data were used for further analyses. The oldest male was estimated to be 32 years old, while the oldest female was 35 years old (Figure 7).

Males and females growth rates were not significantly different (ANCOVA, P>0.05). Females reached a larger maximum size (L_∞= 199.6 year) with a slightly lower growth coefficient (k = 0.04 year^{-1}) than males (177.6, k = 0.06 year^{-1}).

The longevity estimates were 76.84 years for females and 51.28 years for males. The calculation of M from the various methods produced estimates ranging from 0.59 to 0.62 of females and 0.48 to 0.52 of males.

A total of 180 males were captured and

analysed. All individuals with TL of up to 110 cm had uncalcified claspers (subadults) and clasper size varied between 13.3 and 30 cm (mean=16.85, SD=1.12, n=145), but of 135 cm TL forward specimens with calcified claspers and clasper size varied between 26 and 32.5 (mean=29.56, SD=2.48, n=15) in mature males (Figure 8).

Both testis were functional and no significant difference was detected between mean weight of the right and left (paired t-test: t = 1.28; P > 0.05).

There were two phases in the relationship between gonad mass and TL. Up to 110 cm TL, testes mass varied between 9.6 and 22.7 g; with seminal vesicles width varied from 3.5 to 6.3 cm. In the second phase, the gonad mass varied between 21 and 25 g at TL ranging from 135-150 cm, and seminal vesicles width varied from 5.5 and 6.7 cm a TL ranging from 135-150 cm (Figure 8a, b, c).

The number of alar thorn rows varied from 1 to 5 on the right pelvic (mean=3.2, SD=1.8, n=180) and from 3 to 4 on the left one (mean=2.8, SD=2.1, n=180) (Figure 8). There was no significant

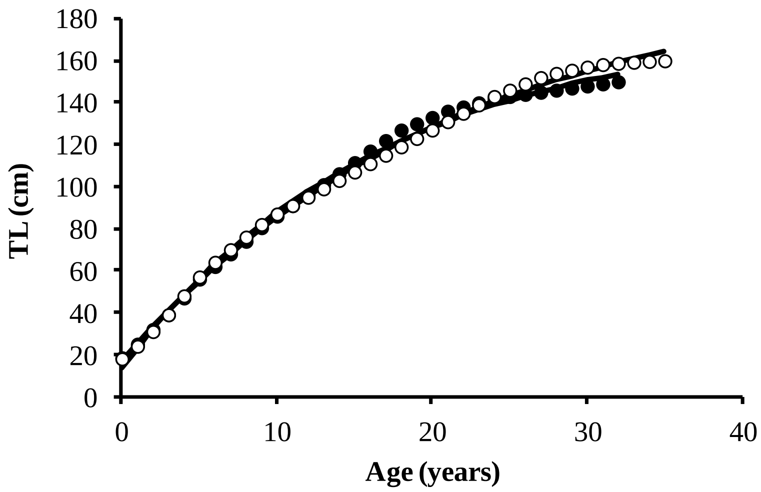

Figure 7. Von Bertalanffy growth curve for females (●*)* and males (○).*Rostroraja alba*, from the Gulf of Gabès.

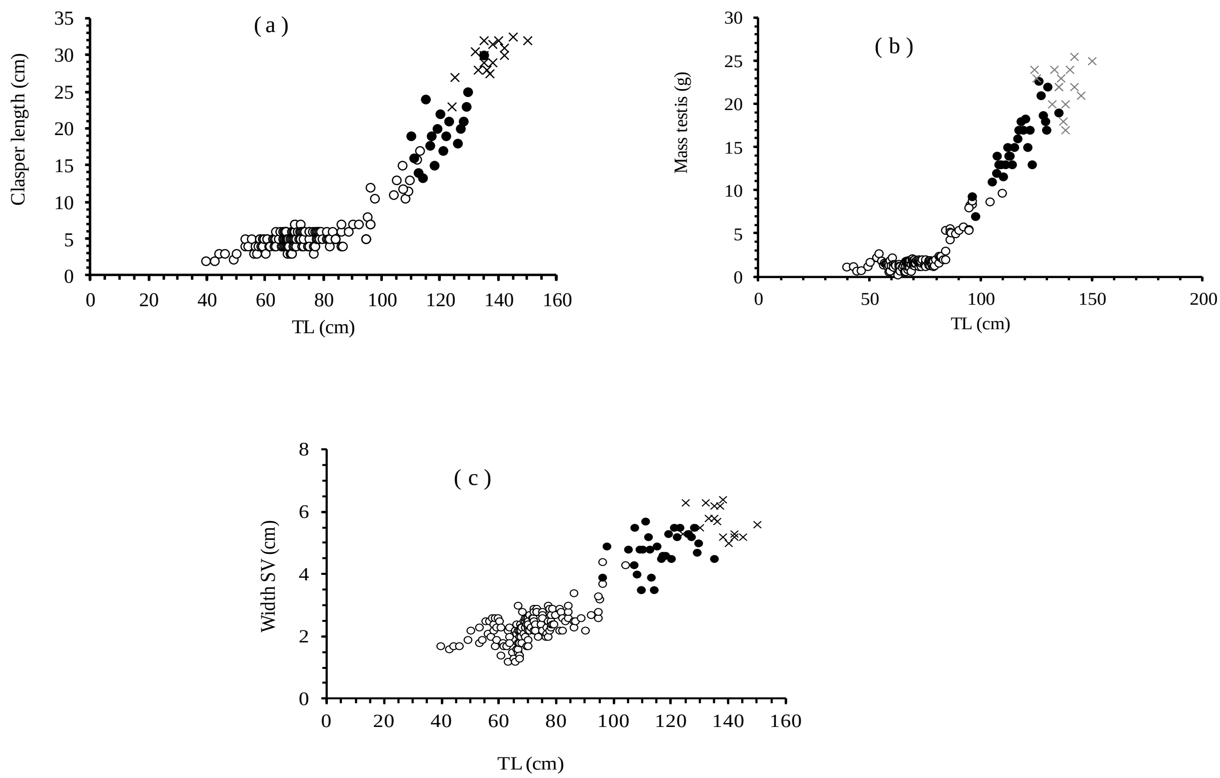

Figure 8. Relationship between total length and (a) clasper length, (b) total testis mass, (c) width of seminal vesicls and (d), *Rostroraja alba*.

difference between the number of alar thorns on the right and left pectoral fin (t=0.34, n=180, P<0.05). Alar thorns were not developed (immature specimens) until 105 cm TL, and only at 110 TL cm the thorns begin to appear.

Additional analysis revealed the smallest sexually mature male within the measured 110 cm TL and the largest juveniles one was 135 cm.

Females had symmetrical gonads and both ovaries are functional. No differences between mean masses gonads (t =0.13, P=0.089), right (mean 14.28) and left (16.4) and diameters follicle (mean right and left: 1.57 and 1.35 cm, respectively; (t = 0.62, P=0.63) were found of mature females.

A total of 140 females were analysed. Gonads grew with TL in two phases (Figure 9a). First, gonad weight varied between 1.2 and 6.72 g (mean=3.24, SD=1.2, n=115) increased slowly with TL, in females with white, non-vitellogenic follicles in the ovaries. At TL=120 cm, an abrupt growth of the gonad weight varied between 25 and 176.5 g (mean=101.06, SD=5.2, n=16), with occurred from TL=145.0 cm began because of the occurrence of the first females with maturing vitellogenic follicles, which represents the onset of vitellogenesis. Females with vitellogenic follicles as well as egg-bearing individuals occurred from TL= 145.0 cm.

White follicles with sizes of 1.4-2.9 mm (mean=2.20, SD=0.15, n=16) occurred in females of up to 145 cm TL. Vitellogenesis began when the follicles reached a diameter of 2.1 cm, and the smallest female with vitellogenic follicles was 120 cm long. As ovulation in egg-bearing females occurs immediately after egglaying in many skates, it was assumed that follicles with diameters ≥2.0 cm were preovulatory (Figure 9b).

Nidamental gland width varied between 0.75 and 2.6 cm in juveniles (mean=1.5 cm, SD=0.51, n=115), and between 3.8 and 5.5 cm in matures. There was high overlapping in the range of nidamental gland width among, juveniles, subadults and adults females in relation to TL (Figure 9 c).

Uterus width varied highly with TL, juveniles females, uterus width varied between 1.5-5.8 cm (mean=3.07 cm, SD=0.42, n=115), while in mature females uterus had a width of 1.5-3.5 cm (mean=1.55 cm, SD=0.57, n=9). Uteri of egg-bearing females varied between 15.3-15.7 cm wide (mean=15.45, SD=0.44, n=9), being the size variation due to the presence of females with term egg capsules and females starting to secret capsules (Figure 9 d) .

One to two egg capsules were commonly observed inside actively spawning females (one in each oviduct). Among all actively spawning females (n = 9), eight females contained two egg capsules in each oviduct. Egg capsules were rectangular-shaped and had two horns at each end.

Furthermore, egg cases length with horns measured between 18.8 and 22.94 cm (20.25±0.83 cm), and between 14.5 and 18 cm (16.64±1.7 mm) in

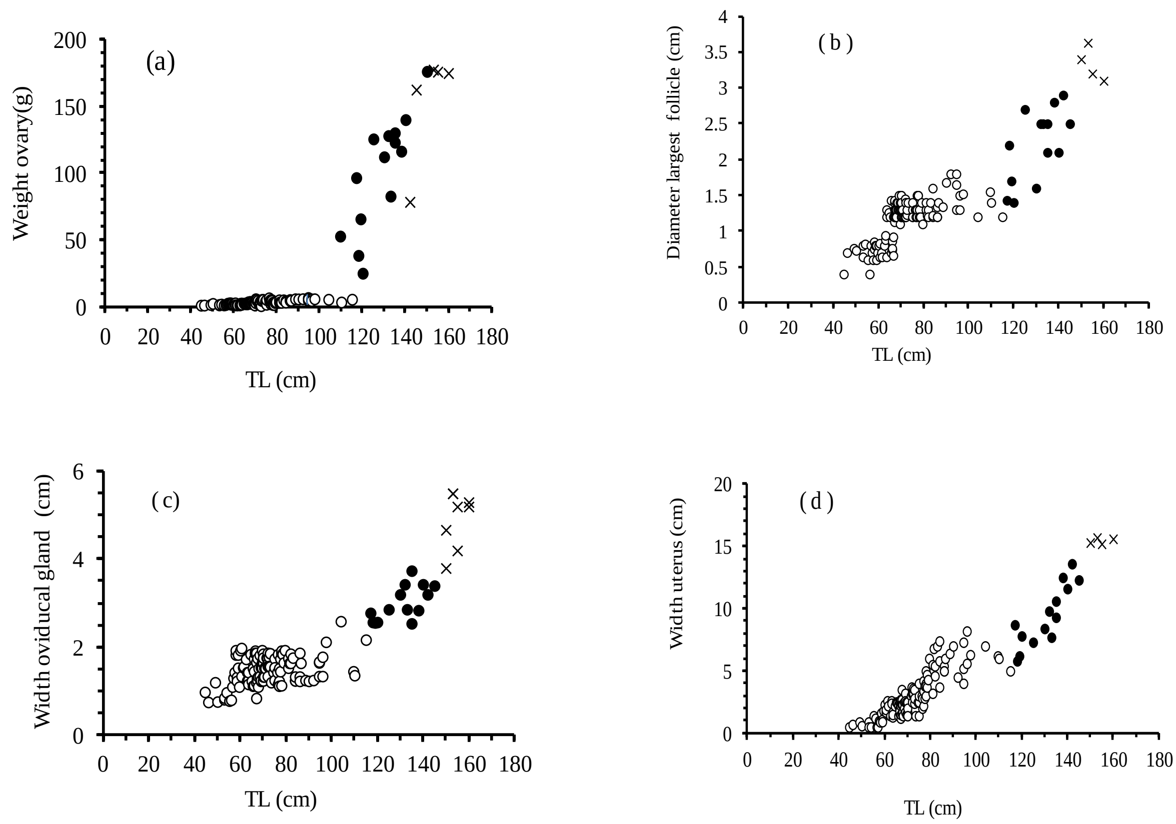

Figure 9. Relationship between total length and total ovary weight (a), diameter of largest follicles (b) width of oviducal gland (c) and width of uterus (d) of thorny skate, *Rostroraja alba*.

length without horns, The width ranged between 7.5 and 8.8 cm (7.25±1.2mm) and in mass between 16.5 and 27.5 g (22.7±0.8 g) (Figure 10).

In males, the IG varied significantly throughout the year (ANOVA, Tukey's post hoc, $P<0.05$), but no significant differences in the IH (ANOVA, Tukey's post hoc, $P<0.05$) (Figure 11a).

In females, the monthly variation of the IG was also statistically significant (ANOVA, Tukey's post hoc,$P<0.05$). The IH also varied significantly throughout the year (ANOVA, Tukey's post hoc, $P<0.05$) (Figure 11b).

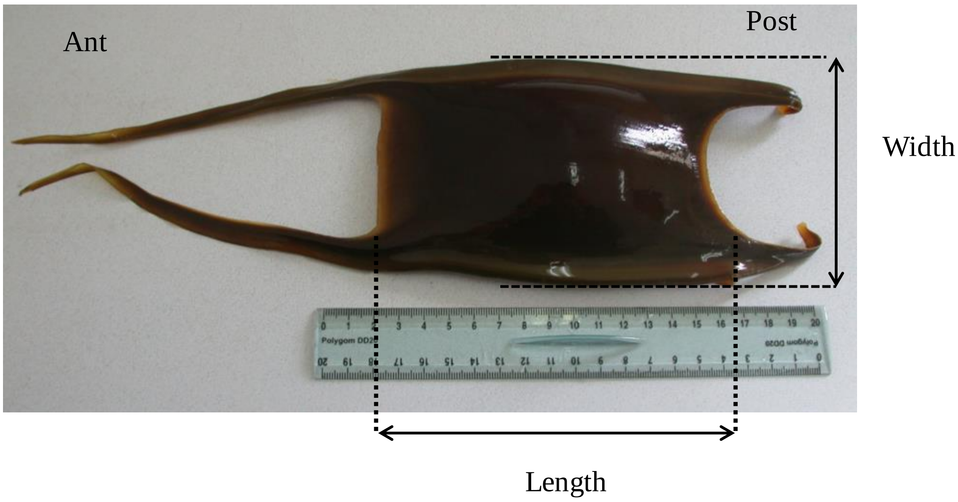

Figure 10. Egg capsule measurements from *Rostroraja alba*.

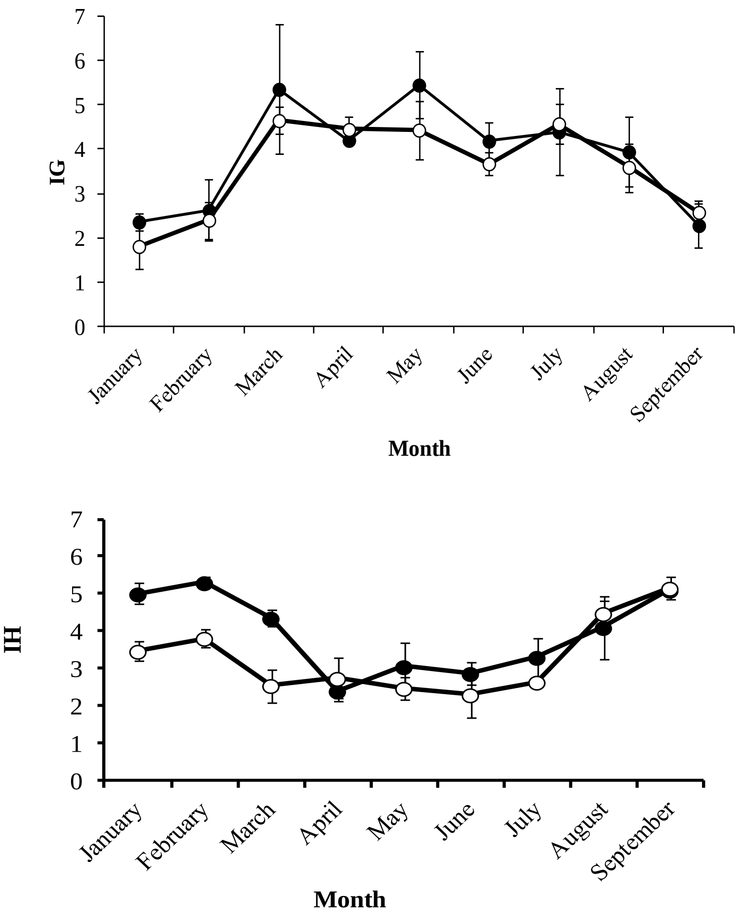

Figure 11. Monthly gonadosomatic index (GSI) for males (●*)* and females (○) (a); monthly hepatosomatic index (HSI) for males and females (b) *Rostroraja alba*.

The smallest specimens attaining sexual maturity were 110 cm TL for males and 120 cm TL for females. All specimens larger than 135 cm TL for males and 145 cm TL for females were mature. Size at 50% sexual maturity was 119.3 cm TL for males and 129.4 cm TL for females (Figure 12). First maturity was estimated to occur at 79.53% and 80.87%, respectively of the maximum TL recorded in this study.

Nevertheless, changes in all the reproductive variables with TL, presence/absence of vitellogenesis in ovaries and egg production were used all together as criteria for classifying males and females as mature or immature.

The oldest juveniles female and male were respectively 30 and 27 years, while the youngest mature female and male were respectively 17 and 13 years. The A50% was estimated to be 23.87 years for females and 19.69 years for males (Figure 13).

Discussion

In the Gulf of Gabès, the white skate was caught especially during spring and summer. Males *Rostroraja alba* attained a smaller maximal size than females confirming the sexual dimorphism in skates (Ebert, 2005).

TL and TM relationships were significantly different between the sexes. But Ebert *et al.* (2008) did not reveal significantly difference of this species. The difference between sexes was found for several skates (Mabragana and Cousseau, 2004; Ruocco *et al.*, 2006). Females reach larger body sizes than males as a consequence of body space needed for egg production and storage during the reproductive stage (Ruocco *et al.*, 2006).

The Analysis marginal increment for white skate indicated that band formation occurs once a year during summer in the Gulf of Gabès. This annual band pair deposition was verified through edge and marginal increment analyses. The MIA is the most commonly employed validation technique among chondrichthyans age and growth studies (Cailliet and Goldman, 2004; Goldman, 2004).

No age validation of this species was done. However, based on previous studies of other

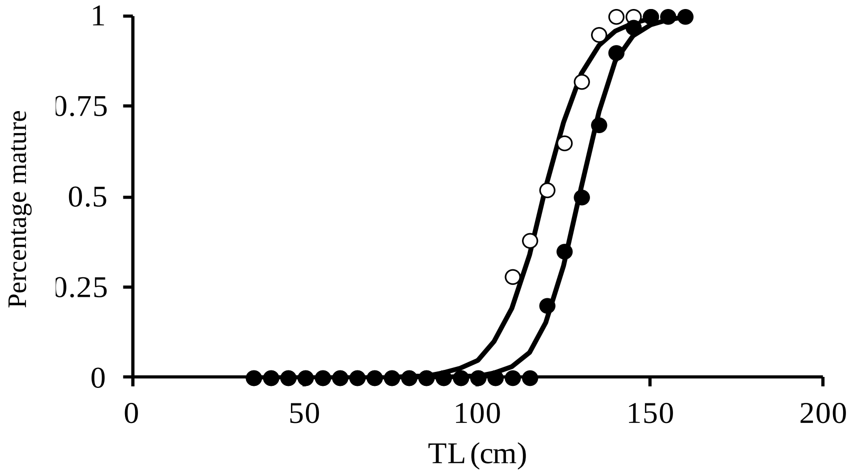

Figure 12. Relationship between the percentage of mature *Rostroraja alba* and total length (TL) for females and males.

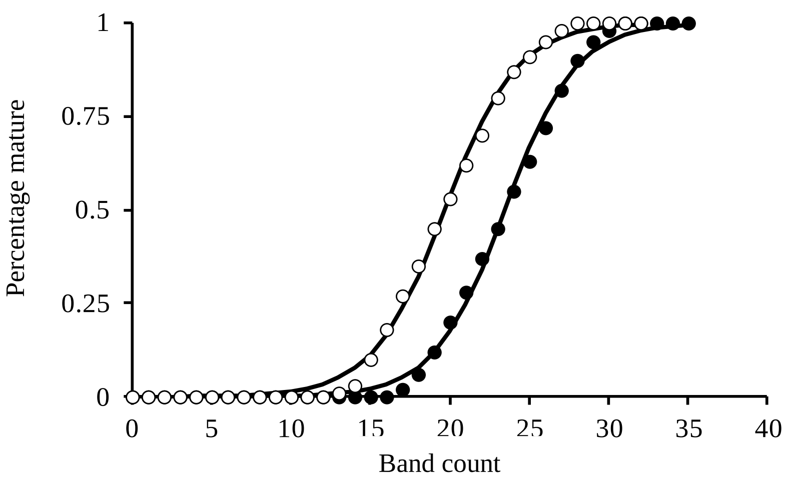

Figure 13. Age at maturity for female and male *Rostroraja alba.*

elasmobranchs, including the longnose skate, we believe that one opaque and translucent band pair is deposited annually. Kadri *et al.*(2012); Kadri *et al.* (2013 a,b) performed MIA on the *Raja miraletus, raja radula* and *raja clavata* off the Gulf of Gabès and found annual periodicity of band pair formation.

In the present study, the relationship between TL and vertebral radius revealed that vertebral growth significantly increased with somatic growth. The marginal increment analysis demonstrates that vertebral growth patterns are a reliable indicator of age in the big skate.

In this study, L_∞ values showed that adult females grew larger than adult males, while the value of K for males was higher than that for females this results are agreeing with previous reports for other rajid species (Walmsley-Hart *et al.,* 1999; Licandeo *et al.,* 2006).

The growth parameters for *R. alba* are consistent with the assumption that larger batoids live longer and grow slower than smaller batoids.

In the present work, the natural mortality rate for white skates was estimated to 0.17. Since the big skate *Rostroraja alba* have not been previously aged in the Mediterranean, this study presents the first estimates of natural mortality.

In fact, estimates for females are considered more relevant for population modeling, because aspect of female population dynamics, including age specific and total life-time fecundity and mortality, determine the potential increase in population numbers (Bishop *et al.,* 2006). Species with mortality levels of a comparable magnitude are ranging from 0.18 to 0.28 for males, and from 0.14 to 0.25 for females of *Bathyraja parmifera* (Mary Elizabeth Matta *et al.,* , 2007) and (M = 0.17) for longnose skate (Christopher, 2007), this study presents the first estimates of natural mortality based on maximum age from this region.

The differences could be truly geographical in nature, or they could be the result of differences in sampling or age estimation criteria between the studies

Body size can be a predictor of vulnerability since most skate fisheries select for larger individuals, and many skate species mature at a large body size (Dulvy and Reynolds, 2002).

The majority of studies in skates on the timing of sexual maturity have used gross morphological changes in female reproductive tracts and structural modifications in male claspers to determine when certain species reach adulthood (Zeiner and Wolf, 1993; Walmsley-Hart *et al.,* 1999; Francis *et al.,* 2001; Ebert, 2005; Neer and Thompson, 2005).

Monthly variations found in the gonadic and hepatic indexes of males and females of *R. alba* indicated that they appear to be reproductively active throughout the year. This result was well confirmed by the fact that females are carrying fully developed oocytes and egg cases throughout the year. Similar to the observations of Alexander *et al.* (2008) for *Bathyraja brachyurops* and *B.* griseocauda and of R. Licandeo *et al.* (2007) for *Dipturus chilensis*.

The hepatic reserves can be used in producing gametes in female oviparous elasmobranchs (Craik, 1978).

Like in other elasmobranchs, an abrupt increase in ovary mass, follicle size and shell gland width in females and Clasper length relative to TL and developement width of vesicles seminales and an increase in testis mass in males has been shown provided a good surrogate to assess maturity in this species (Francis *et al.,* 2001; Conrath and Musick, 2002; Ebert, 2005; Sulikowski *et al.,* 2005a).

In this study, females matured at significantly larger sizes and older ages than males. Sexual dimorphism in terms of size-at-maturity is common in elasmobranch fishes, with females usually maturing later and at larger sizes than males (Nottage and Perkins, 1983; Mabragan and Cousseau, 2004; Kadri *et al.,* 2012; Kadri *et al.,* 2013 a,b). For female elasmobranchs, an abrupt increase in ovary mass and follicle size has been shown to mark the onset of sexual maturity (Francis *et al.,* 2001; Conrath and Musick, 2002; Loefer and Sedberry, 2003; Ebert, 2005; Sulikowski *et al.,* 2005a).

The life history parameters of this population, as in the case of other populations of slow growing, late maturing and low fecundity fishes, render it extremely vulnerable to overfishing even at low levels of fishing effort (Musick, 1999).

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