Research Article

Life-history traits of the commercial blonde ray, *Raja brachyura*, from the central-western Mediterranean Sea

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Abstract

In the present study the life history traits of *Raja brachyura* from Sardinian waters (central-western Mediterranean) were investigated and the results compared with previously published data. The age, growth and reproduction were studied using 1792 specimens. Females ranged from 13.1 to 105.5 cm in total length (TL) and males from 18 to 96.5 cm TL. This species was sexually dimorphic, with females attaining a larger size than males. Both females and males showed positive allometric growth, and significant differences in the relationship between length and weight among sexes were found. The von Bertalanffy growth function (3 parameters), which provided the following values: L_∞=111.14 cm TL, k=0.10 and t_0=-1.3 for females, and L_∞=108.81 cm TL, k=0.11 and t_0=-1.2 for males, was the best-fit age model. *R. brachyura* showed a relatively slow growth rate with males growing slightly more rapidly than females. Length and age at maturity were 87.2 cm (14 years) for females and 80.8 cm (10 years) for males corresponding to 83 and 83.7% of maximum observed length respectively, indicating that the blonde ray is particularly sensitive to fishing pressure. This ray exhibited a restricted reproductive cycle from late May to August, as confirmed by the GSI values. Ovarian fecundity reached a maximum of 44 yolked follicles. Given the relative abundance in Sardinian seas, it is hoped that these results will prove useful for the implementation of basic management measures in order to ensure the sustainability of catches of this species in the Mediterranean Sea.

Keywords: *Raja brachyura*, growth, age, maturity, reproductive cycle, central-western Mediterranean.

Introduction

Skates (Rajidae) are often considered vulnerable to overfishing because they are long-lived, slow-growing, late to mature and produce few young, which, coupled with their generally large size, morphology and aggregating nature, renders them susceptible to capture in many fisheries (Ellis *et al.*, 2010). These features also make them inadequately resilient to fishing mortality, inclined to rapid stock depletion, and unable to rebound quickly from population reductions (Smith *et al.*, 1998; Stevens *et al.*, 2000; Myers & Worm, 2005; Dulvy *et al.*, 2008).

The availability of updated life history information is essential to any successful fisheries management plan. In fact, knowledge of the age, growth and reproductive biology of elasmobranchs is essential for effective management, as population demography or stock assessments require accurate growth, maturity and reproductive potential data, and also other biological knowledge, such as the gathering of length-weight and disc-length conversion factors (Walker, 1998; Ebert *et al.*, 2008).

The Mediterranean Sea represents a hotspot of marine biodiversity that is exposed to multiple threats, including fishing pressure, habitat loss and degradation, pollution, eutrophication and, more recently, climate change and invasion by alien species (Coll *et al.*, 2010). In the region, elasmobranchs are represented by 85 shark and batoid species (Bradai *et al.*, 2012) and have been heavily exploited, with more than 40% of the species evaluated as being under threat (Cavanagh & Gibson, 2007; Bradai *et al.*, 2012). Cartilaginous fish currently represent a fishery by-catch in the Mediterranean Sea, although some species have an important commercial role (Castro *et al.*, 1999; ICCAT, 2001).

The blonde ray *Raja brachyura* Lafont, 1873 is a benthic species that shows a clear preference for sandy bottoms in the superior continental shelf (Serena, 2005). It is distributed in the north-east Atlantic from Norway to Morocco and is more common in the western-central than the eastern Mediterranean Sea (Ellis *et al.*, 2005; Serena *et al.*, 2010). Generally, it is considered an uncommon species in the Mediterranean (Matallanas, 1974; Serena, 2005), but the last observations of Follesa *et al.* (2003, 2010), Catalano *et al.* (2007) and Ragonese *et al.* (2003) indicate that the Sardinian coasts and western Sicily represent a zone where *R. brachyura* is relatively abundant. However, the IUCN red list classified it, globally, as *Near Threatened* (Ellis *et al.*, 2009) but, specifically for the Mediterranean

Sea, as *Data Deficient* (Cavanagh & Gibson, 2007).

The blonde ray is a commercially important species and is caught and landed across its range (Catchpole *et al.*, 2007). It is sometimes targeted in areas where it is locally abundant but is normally taken as by-catch in mixed demersal fisheries using trawls, gill nets and long-lines (Gibson *et al.*, 2006).

Studies on the biology of this species have been carried out mainly in the north-eastern Atlantic Ocean. They have focused on growth rates and ageing (Holden, 1972; Fahy, 1991; Gallagher *et al.*, 2005; Serra-Pereira *et al.*, 2005a), conversion factors (Dorel, 1986; Serra-Pereira *et al.*, 2010; McCully *et al.*, 2012), some reproductive aspects (Clark, 1922; Holden *et al.*, 1971; Gallagher *et al.*, 2005; McCully *et al.*, 2012) and diet (Holden & Tucker, 1974; Quiniou & Rabarison Andriamirado, 1979; Ajayi, 1982; Ellis *et al.*, 1996; Farias *et al.*, 2005). However, information on its general biology in the Mediterranean is very limited. Recently, only Catalano *et al.* (2007) provided information on reproduction, population structure and feeding habits and Follesa *et al.* (2010) on feeding, relative to the specimens caught in Sardinian seas (central-western Mediterranean).

Based on the need to update life history information for this commercial skate species, this work provides the first data on age and growth estimates and gives additional information on maturity in the Mediterranean Sea. Specifically, we focused on: (1) presenting detailed information on age and growth rates using different growth models; (2) quantifying the timing and duration of maturation, mating and egg laying seasons; (3) characterizing the reproductive cycle based on morphological changes in reproductive organs during maturation; and (4) estimating maturity ogives for both sexes. The results were also compared with previously published data in order to examine whether this species may exhibit regional-specific life-history strategies within European waters. The estimation of these parameters could be important for future management of this commercially exploited and important species in the Mediterranean Sea.

Materials and Methods

Sampling

Specimens of the blonde ray *R. brachyura* were caught during experimental trawl surveys and commercial hauls performed from 2005 to 2013 in Sardinian waters at depths from 28 to 175 metres (Fig. 1).

For each individual, the total length (TL) and disc width (DW) was measured in centimetres; the clasper length (CL) in males and the oviducal gland width (OGW) in females were recorded in millimetres using a gage. Total mass (TM) and gonad mass (GM) were recorded in grams. Specimens were sexed and the maturity stages were determined following the scale of oviparous elasmobranchs proposed in the Medits handbook (MEDITS, 2012), which
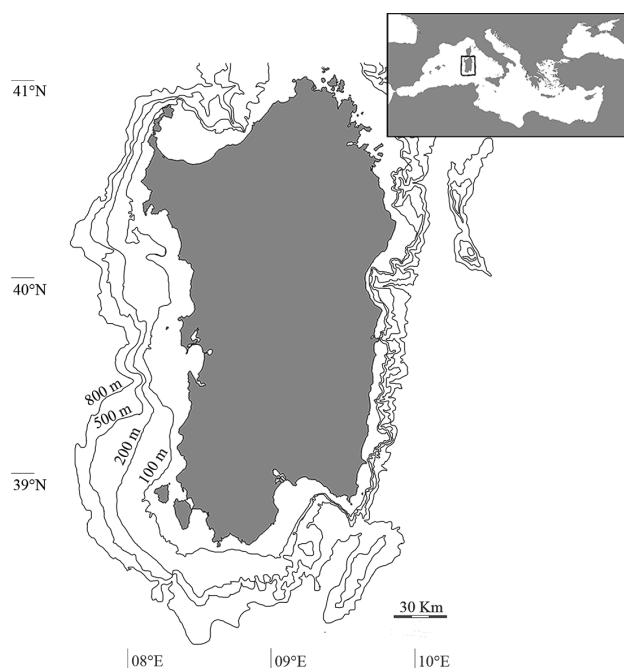
Fig. 1: Map of the study area in which specimens of *Raja brachyura* were caught.

is in agreement with the maturity scale adopted by the ICES for the north-eastern Atlantic (ICES, 2010). According to this scale, females were classified in six stages: stage 1, immature/virgin; stage 2, maturing; stage 3A, mature; stage 3B, mature extruding; stage 4A, resting; stage 4B, regenerating and males in five ones: stage 1, immature/virgin; stage 2, maturing; stage 3A, mature; stage 3B, mature active; stage 4, resting.

Data analysis

The sex-ratio (SR, males:females) was calculated for the samples. The significance of deviation from the 1:1 null hypothesis was tested by the chi-square test (χ^2).

The Kolmogorov-Smirnov (KS) two-sampled test was used to find statistically significant differences in the length frequencies by sexes (Zar, 1999).

A sex-disaggregated length-weight relationship was fitted: $TM = a\,TL^{\,b}$.

The function was log-transformed so that a linear regression could be fitted. The linear relationship between TL and WD was calculated according to the equation: $WD = a\,TL + b$.

Student's *t* test was used to test the equality of regression coefficient between the linear regression equations (Zar, 1999) of males and females.
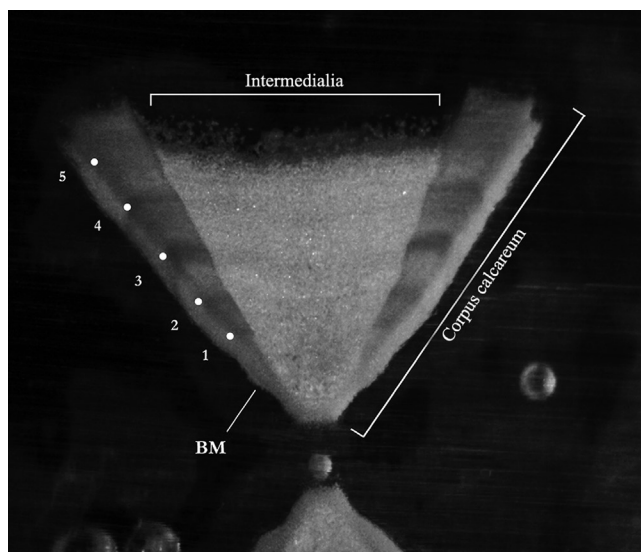
Age and growth - Preparation of ageing structures

A minimum of 10 vertebral centra were extracted from each thoracic cavity of a subsample of 168 blonde rays (76 females and 92 males) ranging from 15.6 cm to 99.5 cm TL. The neural and haemal arches were dissected with a scalpel, and then each centrum was soaked in a 5% sodi-

Medit. Mar. Sci., 16/1, 2015, 90-102

91

um hypochlorite solution in order to remove the remaining connective tissue (Goldman, 2005). Soaking time ranged between 5 and 20 minutes. Cleaned samples were photographed using a Zeiss Stemi 2000-C stereomicroscope (0.65X – 5X) with annexed Pixelink microscope camera (PL-A686C), so as to measure the vertebral radium (VR) in millimetres using the tpsDig2 software (Rohlf, 2005). The ANOVA test was performed to test for significant differences in VR to TL between males and females. Cleaned vertebra were embedded in biphasic epoxy resin (Struers Caldo-fix 2), mounted on a slide and ground using a polisher (Struers mod. DAP-V) with progressively fine abrasive disks (320, 500, 800 grit). Final sections were no greater than 0.5 mm in thickness. A subsample of vertebral centra extracted from 5 individuals were stained with silver nitrate (Caillet *et al.*, 1983; Kusher *et al.*, 1992) and Alizarin red (LaMarca, 1966) after sectioning in order to determine which treatment allowed the best visibility of bands. Each vertebral section was photographed as previously described. The images were post-processed converting them to grey-scale and the contrast and clarity were increased following the technical manual of Campana (2013), in order to enhance the vertebral growth bands.

Age determination

Vertebral sections were read independently by two operators without any prior knowledge of the sample's gender or size. Readings were repeated three times per section. The birth mark (BM) (Fig. 2) was defined as the first clear mark that corresponds to an angle change in the corpus calcareum and annuli were defined as an opaque and translucent band pair (Casey *et al.*, 1985; Sulikowski *et al.*, 2003).

Growth models were adjusted to length-at-age data from vertebrae with 4 out of 6 consistent readings, with differences inferior to 2 years (Sulikowski *et al.*, 2003), all the others were discarded. To determine the accuracy of the age readings, the Index of Average Percent Error (IAPE) was calculated (Beamish & Founier, 1981):

$$\text{IAPE} = \frac{1}{N} \sum \left[\frac{\frac{1}{R \; \Sigma(X_{ij}-X_j)}}{X_j} \right] \times 100$$

Where N represents the number of samples aged; R is the number of readings; X_{ij} is the ith age determination of the jth fish; and X_j equals to the average age calculated for the jth fish. The % Coefficient of Variation (% CV) (Chang, 1982) and the Percentage of Agreement (PA) using the Eltink (2000) Excel workbook, were also calculated. The bias test was used according to Eltink (2000) to evaluate the likelihood of bias between readers.

Age estimation

Length at age analysis was performed, using the FISAT II software (Gayanilo *et al.*, 2005), in order to fit the three von Bertalanffy Growth Function (3VBGF) parameters (Von Bertalanffy, 1938): where TL is the theoretical maximum attained length, TL is the length at the age or time, k is the growth coefficient and t_0 represents the hypothetical age of an individual at length 0. In addition, the two VBGF (2VBGF) parameters proposed by Fabens (1965) were also fitted to the length at age data, according to the following equation: $\text{TL} = L_\infty \, (1 - be^{-kt})$, where $b = (L_\infty - L_0) \, L_\infty^{-1}$ and L_0 is the length at birth. To determine which function provided the best fit, the root mean square error (RMSE) (Fisher *et al.*, 2013) and the Akaike Information Criterion (AIC) (Akaike, 1974; Haddor, 2001) were calculated. AIC was expressed as: $\text{AIC} = n \times \ln(\text{MSE}) + 2k$ where n is the total number of samples, MSE is the mean square of residuals and k is the number of the growth function's estimated parameters.

Growth functions were calculated for combined sexes and also for females and males separately. The ANCOVA test was performed to test differences between the growth of females and males.

Maturity

The measurements taken from the reproductive organs of both sexes were analyzed by maturity stage in order to characterize the maturation process. The null hypothesis of no significant differences between maturity stages in the OGW, in females, and in clasper length, in males, was tested using ANOVA.

The non-parametric Kruskall-Wallis test was used to find significant differences between lengths and between ages within each maturity stage, both for males and females.

First maturity was considered to be the size (TL) of the smallest mature skate examined for each sex. Size at maturity (L_{50} = length at which 50% of the individuals are mature) was estimated, for males and females separately, by fitting maturity ogives (using data restricted to the reproductive season) to the proportion of mature individuals in each 5 cm TL size class. Non-linear least squares regres-

Fig. 2: A vertebral section of a five-year old *Raja brachyura* (TL = 51.7 cm) (BM, birth mark).

92

Medit. Mar. Sci., 16/1, 2015, 90-102

sion was used to estimate the parameters: $P = 100 \, (1 + \exp(a + (b \times TL))^{-1}$ where P is the proportion of mature fish at L_T size class and 'a' is the intercept and 'b' is the slope of the maturity curve. L_{50} is the size at maturity = $(a \times b)^{-1}$. The same procedure was followed to estimate age at maturity (A_{50}), the age at which 50% of the individuals are mature.

Reproductive seasonality was inferred through an analysis of: (1) the seasonal evolution of the percentage of maturity stages of females and males, and (2) the seasonal changes in the gonadosomatic index (GSI = (GM x TM)$^{-1}$×100) for maturing and mature specimens. The seasonal variations found with both methods were compared by analysis of variance (ANOVA).

Ovarian fecundity was defined as the total number of eggs released per female during the egg laying season estimated by the total number of yolked follicles (yellow in colour; mature stage) counted in both ovaries. The diameter (mm) of yolk follicles was also measured.

Results

Size structure

A total of 1792 specimens were caught and measured: 862 were females and 930 males (SR = 0.52, χ^2 = 1.29, $P > 0.05$).

The length of females ranged from 13.1 to 105.5 cm TL with a total mass (TM) of 5.8-9145.0 g, while males ranged from 12.0 to 96.5 cm TL (Fig. 3) with a total mass (TM) of 6.4-7912.0 g. Although, male and female specimens covered generally similar length ranges, the actual distribution of lengths were significantly different at the 95.0 % confidence level (Kolmogorov-Smirnov test, $P < 0.05$).

Length–weight and total length–disc width relationships

The regression slopes of total length and total mass (Fig. 4A,B) between sexes differed significantly (ANOVA, F-ratio = 38285.44, $P < 0.05$). The relationship found in females and males followed the equations:

Females: TM = 0.0012* TL$^{3.4394}$, r^2 = 0.98; Males: TM = 0.0014* TL$^{3.3765}$, r^2 = 0.97.

Total length and disc width were found to be linearly related and statistically different among sexes (ANOVA, F-ratio = 245594.76, $P < 0.05$). The linear relationships found in females and males were, respectively: DW = 0.7216* TL - 0.8588 (r^2 = 0.99) and DW = 0.7104* TL -0.5726 (r^2 = 0.99).

Age and Growth

The relationships between vertebral radium (VR) and total length (TL) (Fig. 5), calculated for females (b = 0.052; a = -0.449; r^2 = 0.97) and males (b = 0.0454; a = -0.1947 r^2= 0.99) respectively, showed significant differences between sexes (ANOVA, F-ratio = 2200.37; $P < 0.05$; r^2 = 0.97).

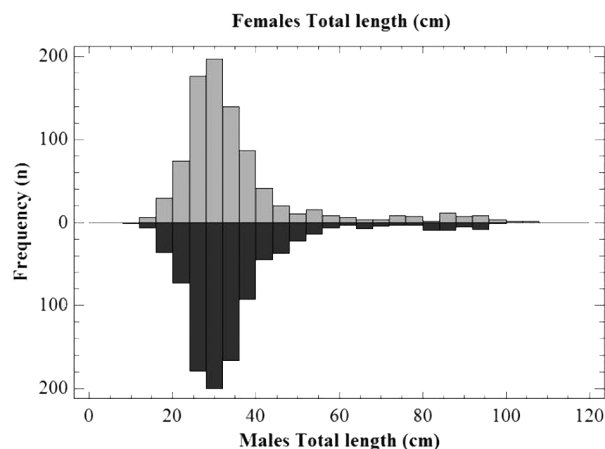A clear increase in band visibility was not observed in either of the staining methods (silver nitrate and aliza-

Fig. 3: Length distribution of female and male specimens of *Raja brachyura*.
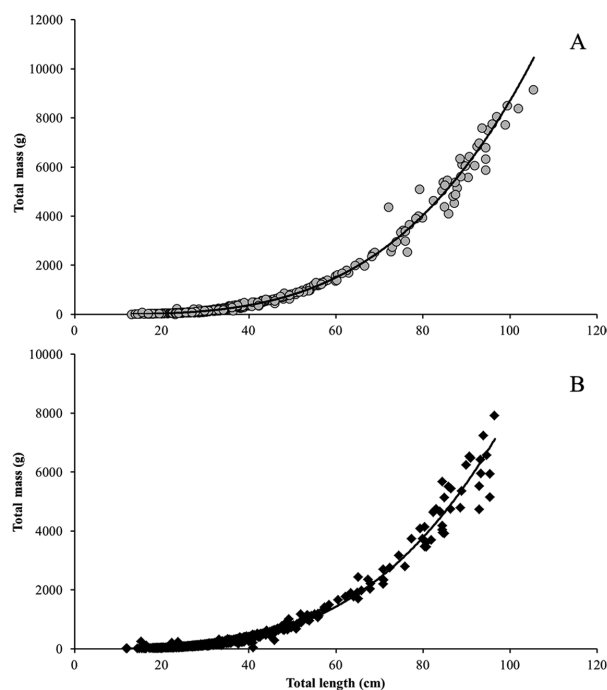

Fig. 4: Relationship between total length and total mass of A) female and B) male *Raja brachyura*.

rine red) utilized on the vertebrae, so the readings were made on unstained samples.

Age readings showed good reproducibility (IAPE = 9.23%; % CV = 12.7; PA = 79.8%). No bias was found between readers (z= -0.847). The mean age ± 2 s.d. versus the modal age for all six reading repetitions combined, is shown in Fig. 6. Growth bands were relatively visible and mostly unambiguous. However, out of the 168 processed vertebrae, 30 (17.8%), not belonging at a specific age or gender group, were discarded because the vertebrae were not read in the same way (at least 4 times out of 6). Of the remaining 138 samples, 79 were males ranging from 18.4 cm to 95.5 cm in TL and aged between 0 and 16 years, and 59 females from 25.2 to 90.8 cm TL with ages between 1 and 15 years. Modal age was 2 for both sexes.
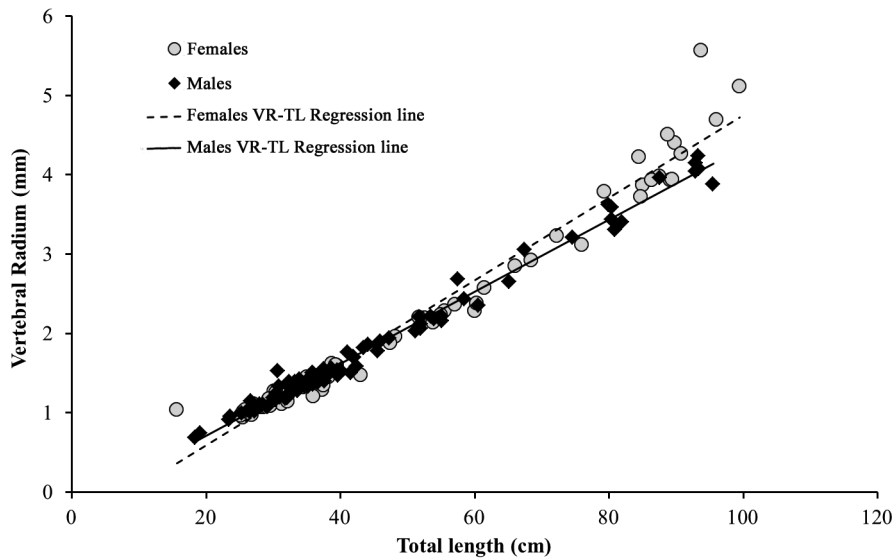
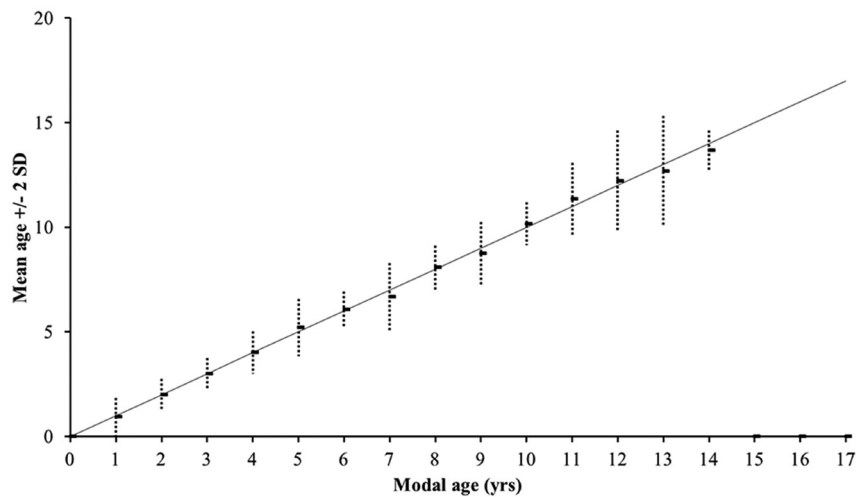***Fig. 5:*** VR - TL relationship for females and males with linear regression lines of *Raja brachyura*.

Fig. 6: Age bias plot with the mean age ± 2 S.D. recorded for all readers combined, plotted against the modal age. The estimated mean age corresponds to modal age if the estimated mean age is on the 1:1 equilibrium line (solid line). Relative bias is represented by the age difference between estimated mean age and modal age.

The ANCOVA test revealed statistically significant differences between genders in length at age data (F-ratio = 1005.185; $P < 0.001$). Von Bertalanffy growth parameters for both 3VBGF and 2VBGF functions for sexes combined and for females and males separately are given in Table 1, and the resulting curves are plotted in Fig. 7. The analysis of RMSE and AIC showed that the traditional 3VBGF provided the best fit to our data for each group, so further analyses were based on this growth function and the mean length at age was calculated (Table 2).

The obtained VBGFs (n = 138) were applied to the entire samples (n = 1792) pool in order to estimate the age structure of the sampled population (Fig. 8). The majority of specimens were younger than six years, and the modal age class for both sexes was 2 years. The Kolmogorov Smirnov test showed statistically-relevant differences in age distribution between the genders ($P < 0.05$).

Maturity

Even though there were some overlaps in lengths and ages of adjacent maturity stages, a clear increment in lengths and ages through the evolution of maturity stages was observed. Immature females ranged from 13.1 to 84.5 cm TL (n = 812) and from 0 to 13 years in age, maturing females from 53 to 92 cm TL (n = 23) and from 5 to 16 years, mature females from 85 to 105.5 cm TL (n = 18) and from 13 to 29 years and regenerating females from 84.8 to 90.8 cm TL (n = 8) and from 13 to 16 years (Fig. 9 A, B). Immature males ranged from 12 to 71.1 cm TL (n = 875) and from 0 to 8 years, maturing males from 45.4 to 80 cm TL (n = 20) and from 4 to 11 years, mature males from 77.5 to 96.5 cm TL (n = 29) and from 10 to

Table 1. 3VBGF and 2VBGF parameters obtained for males, females and combined sexes (3VBGF = 3 parameters von Bertalanffy growth function; 2VBGF= 2 parameters von Bertalanffy growth function); L_∞ = asymptotic maximum total length [± s.e.]; k = growth coefficient ± s.e.]; t_0 = theoretical age at which TL equals zero; L_0 = TL at birth = 12.1 cm; AIC = Akaike's information criterion; RMSE = residual mean square error). s.e. = standard error; na = not available.

Method	n	Max obs. Size (cm)	L_∞ ± s.e.	k ± s.e.	t_0 ± s.e.	L_0(cm)	RMSE	AIC
3VBGF Males	79	95.5	108.81 ± 10.38	0.11 ± 0.021	-1.2 ± 0.31	na	5.343	270.77
3VBGF Females	59	90.8	111.14 ± 10.59	0.10 ± 0.020	-1.3 ± 0.37	na	4.487	183.16
3VBGF Combined	138	95.5	111.34 ± 7.45	0.10 ± 0.014	-1.19 ± 0.23	na	5.050	452.99
2VBGF Males	79	95.5	108.81 ± 10.38	0.11 ± 0.021	na	12.1	5.606	278.38
2VBGF Females	59	90.8	111.14 ± 10.59	0.10 ± 0.020	na	12.1	4.714	188.95
2VBGF Combined	138	95.5	111.34 ± 7.45	0.10 ± 0.014	na	12.1	5.140	457.86
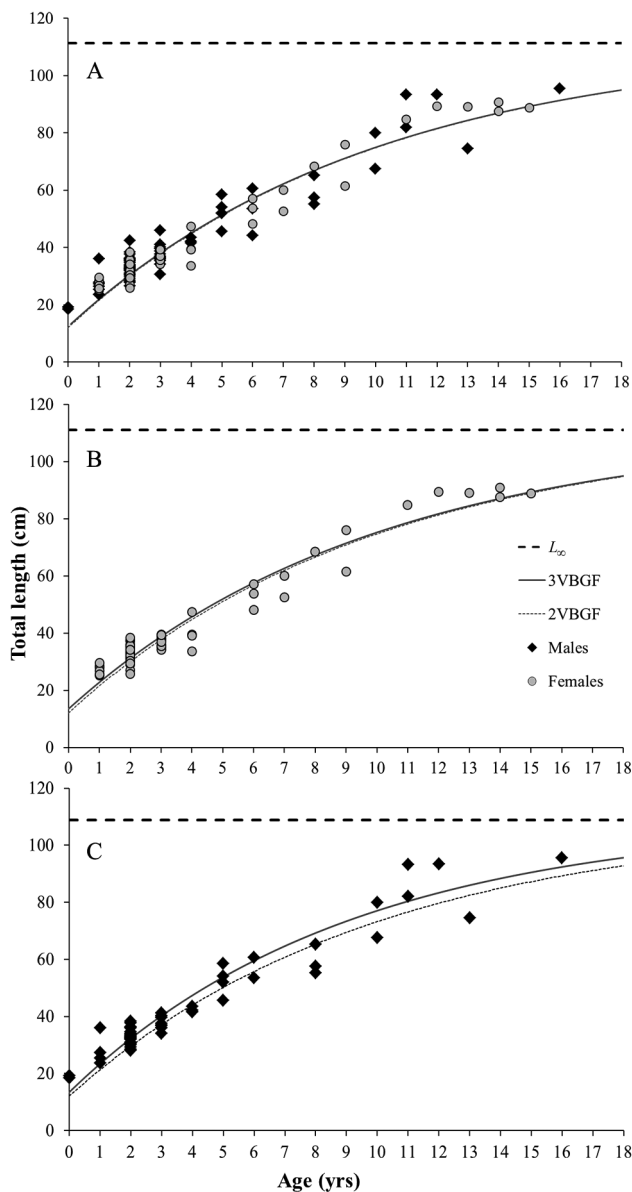
Fig. 7: The three and two-parameter von Bertalanffy Growth Curves (3VBGF and 2VBGF respectively) for combined sexes (A), females (B) and males (C) of *Raja brachyura*.
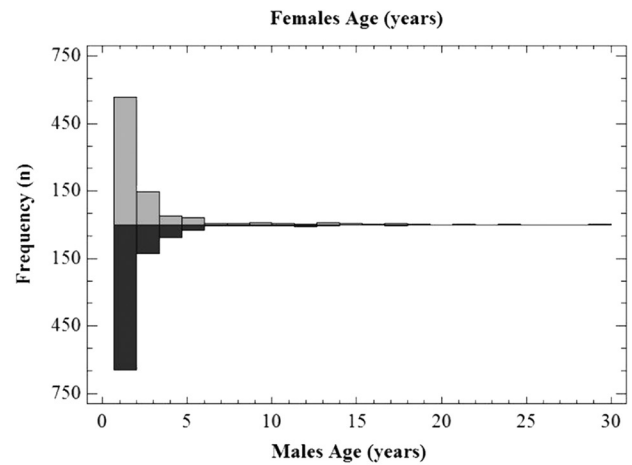

**Fig. 8:** Age distribution of female and male specimens of *Raja brachyura*.

19 years and resting males from 74.6 to 95.5 cm TL (n = 6) and from 9 to 18 years (Fig. 9 a, b). Differences were detected for lengths and ages observed in each maturity stage for both males (Kruskall-Wallis test, $P < 0.001$) and females (Kruskall-Wallis test, $P < 0.001$).

Oviducal gland width (OGW) increased with length and the maturity stage development (Fig. 10A). OG growth appeared slow in immature and maturing individuals, but subsequently growing fast at the mature stages 3A and 3B. There were statistically significant differences between the OGW of all stages except for stage 3A and 3B (ANOVA, F-ratio = 205.6, $P < 0.05$).

Similarly, the relationship between average clasper length and TL showed that initially these organs tend to grow slowly with size, with accelerated growth at the beginning of maturation (at around 80 cm TL) (Fig. 10B). Statistically significant differences were found between the clasper lengths (CL) at all stages, except between Stage 3A and 4 (ANOVA, F-ratio = 1023.54, $P < 0.05$).
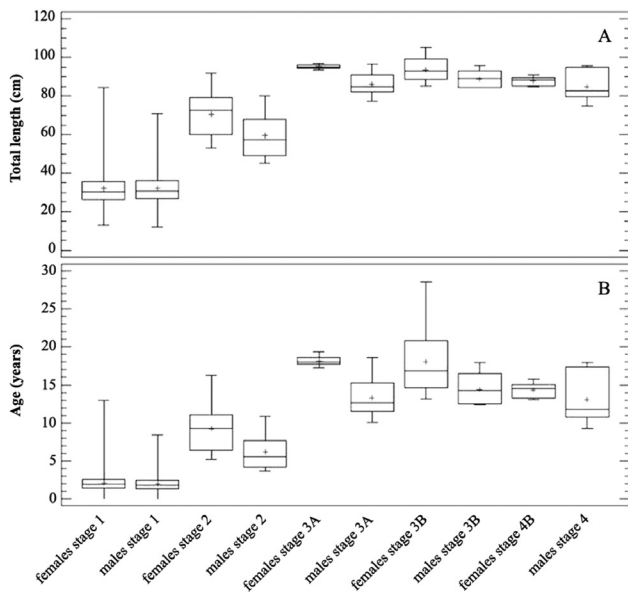
Fig. 9: Box and whiskers plot with the mean, standard deviation, range of length (A) and ages (B) for both males and females at each maturity stage. Stage 1=immature, stage 2=maturing, stage 3A=mature, stage 3B=mature extruding in females and stage 4=resting males.
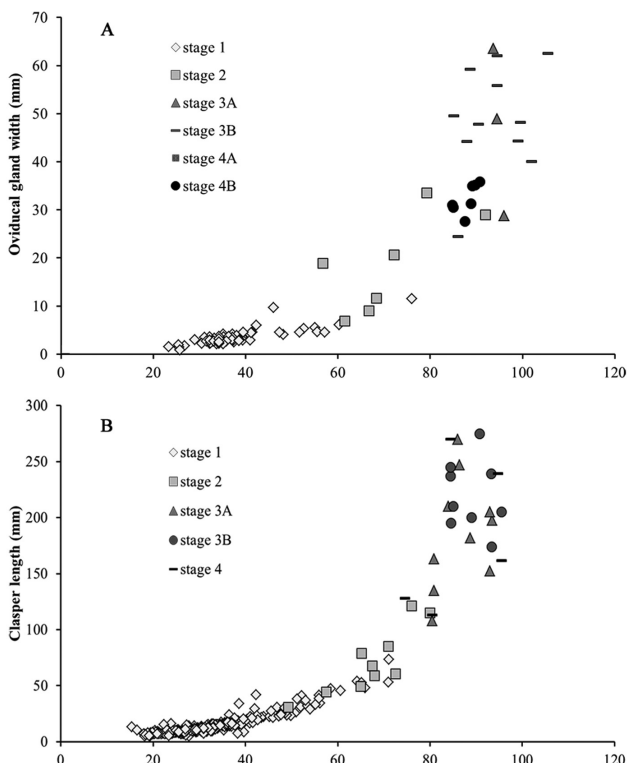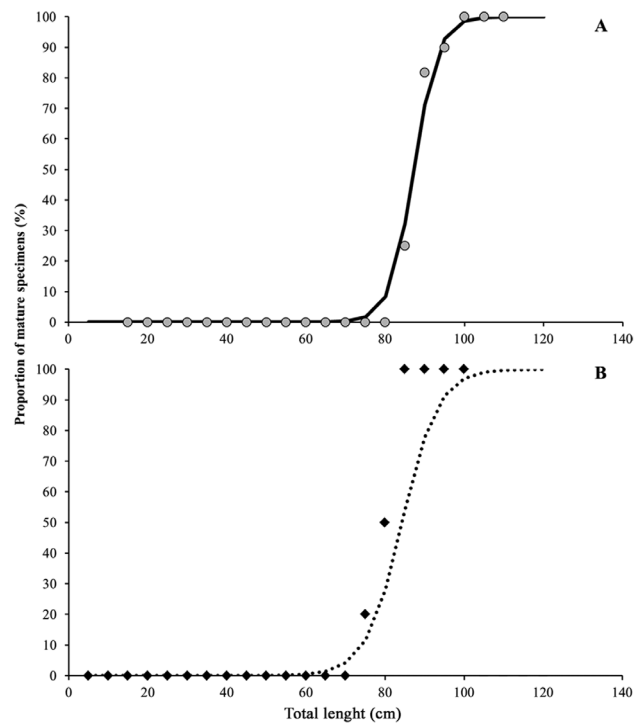

Fig. 10: *Raja brachyura* - Relationship between total length and oviducal gland width in females (A), and clasper length in males (B).

Table 2. Mean length (TL, cm) at age of *Raja brachyura* (S.D., standard deviation)

Age	Mean length	S.D.
0	21.4	3.2
1	28.0	1.9
2	33.0	0.5
3	37.2	0.8
4	43.0	2.0
5	52.6	0.8
6	53.4	1.0
7	57.4	2.8
8	62.6	1.2
9	67.9	6.3
10	73.8	4.3
11	84.4	3.4
12	88.0	2.4
13	-	-
14	88.6	1.6
15	90.4	2.1
16	93.1	3.5

Fig. 11: Maturity ogives in length for female (A) and male (B) *Raja brachyura*.

were 87.2 cm TL (s.e. = 22.8) and 14 years for females and 80.8 cm TL (s.e. 12.9) and 10 years for males (Fig. 11A, B; Fig. 12A,B and Table 4), reaching maturity at 83% and 83.7% of the maximum observed size respectively. The largest immature *R. brachyura* were 80 cm TL (males) and 87.3 cm TL (females).

The relative frequency of each maturity stage by season, for both males and females, is shown in Fig. 13A and B. During the sampling period, all maturity stages were recorded with some variations in their occurrence. In particular, a predominance of immature female speci-

Size at maturity

Females matured later than males with a TL of first maturity at 85 cm for females and 74.6 cm for males. The estimated size at maturity (L_{50}) and age at maturity (A_{50})

96

Medit. Mar. Sci., 16/1, 2015, 90-102
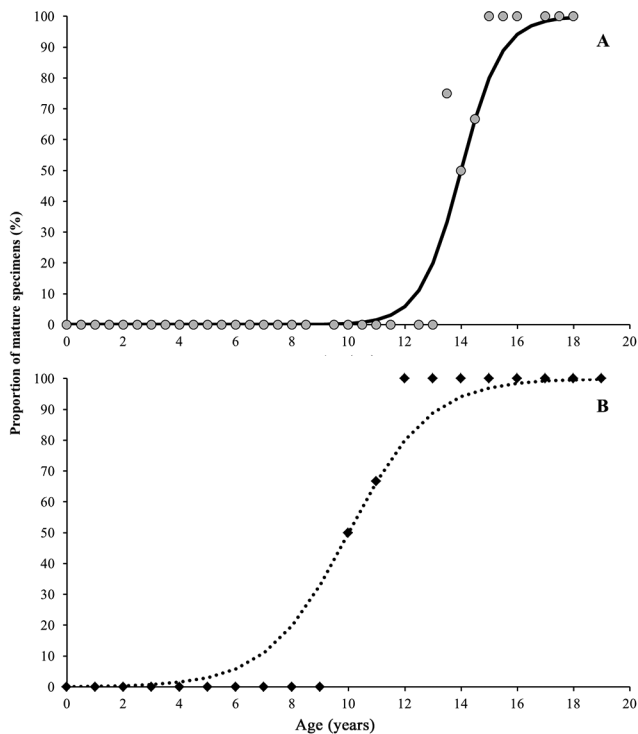
Fig. 12: Maturity ogives in ages for female (A) and male (B) *Raja brachyura*.
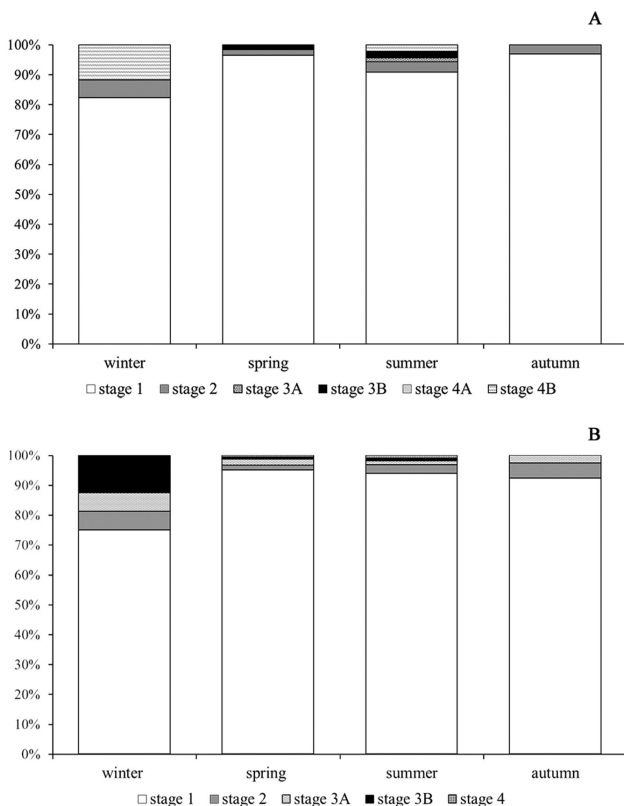
Fig. 13: Seasonal distribution of *Raja brachyura* females (A) and males (B) at each gonadal phase during the sampling period.
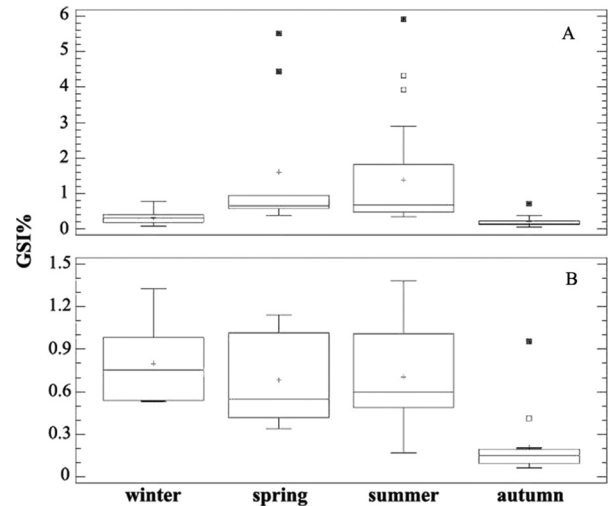
mens was observed throughout the year. For females, the reproductive period extended from late May to summer, although a high frequency of females with egg cases was found mainly during the summer months. Like females,

Fig. 14: Seasonal variations of the gonadosomatic index for both maturing and mature *Raja brachyura* females (A) and males (B).

immature males were predominant in the population. Mature specimens were found in winter, spring and summer.

The GSI analysis of maturing and mature specimens throughout the year showed that females had the highest value during the summer. During the rest of the year, the average GSI remained relatively low, indicating that this species was not in reproductively active phase (Fig. 14A). In males, the GSI values were high from winter to summer, while in autumn very low values were observed even if a mature male was recorded (Fig. 14B).

Fecundity

The ovarian fecundity in mature females (85.1 - 93.7 cm TL) varied from 37 to 44 ripe follicles in both ovaries (mean ± S.D., 41.0 ± 3.6 follicles). The diameter of vitellogenic follicles varied from 9.8 to 44.7 mm with a mean size of 23.6 mm (± 9.08 mm).

Discussion

The present study provides a first estimate of age, growth and reproductive data for *R. brachyura* in the Mediterranean Sea.

The overall length frequency distributions, length-weight relationships and examination of maturity stages indicated that the blonde ray showed sexual dimorphism with females growing larger than males. This pattern has also been observed for the same species by Catalano *et al.* (2007) along the coasts of Asinara Island (Sardinia) and Gallagher *et al.* (2005) in the Irish waters, being a common feature among the Raijdae family (e.g. white-dotted skate *Bathyraja albomaculata* (Norman, 1837), Ruocco *et al.*, 2006; thornback skate *Raja clavata* (L., 1758)*,* Gallagher *et al.*, 2005; long-nose skate *Dipturus oxyrinchus* (L., 1758) (Yigin & Ismen, 2010).

Positive relationships were found between total length

Medit. Mar. Sci., 16/1, 2015, 90-102

97

and total weight with allometric coefficient values higher than 3, showing an allometric growth significantly different between males and females. Similar results were found by Dorel (1986), Serra-Pereira *et al.* (2010) and McCully *et al.* (2012) in the Atlantic Ocean and by Catalano *et al.* (2007) in the Mediterranean Sea for the same species (see Table 4). The same relationship was also observed in other skates from the south-west Atlantic, such as the zipper sand skate *Psammobatis extenta* (Garman, 1913) (Braccini & Chiaramonte, 2002), the smallnose fanskate *Sympterygia bonapartii* Müller & Henle, 1841 (Mabragaña *et al.*, 2002), the shortfin sand skate *Psammobatis normani* McEachran, 1983 (Mabragaña & Cousseau, 2004) and *Bathyraja albomaculata* (Ruocco *et al.*, 2006).

The observation of unstained vertebral centra sections provided good results in terms of band visibility so we suggest the adoption of this method for further analysis on the blonde ray vertebra rather than more elaborate and less time-efficient techniques.

Although our samples for ageing estimation did not permit the verification of the seasonality of band deposition through Marginal Increment Ratio analysis (MIR), the annual pattern has already been confirmed for *R. brachyura* in Irish waters by Gallagher *et al.* (2005). The values of IAPE (9.23%) and % CV (12.7) were good and highly consistent with other elasmobranch studies. Since Campana (2001) reports that % CV from cartilaginous fish studies rarely falls below 10%, we can consider the age estimates of this study as adequate.

When analysing the growth rate of blonde ray, the classic von Bertalanffy growth function (3VBGF) provided a better fit to the observed data than the two-parameter function VBGF (2VBGF). The 3VBGF function is the only method adopted for this species in the literature (Holden, 1972; Fahy, 1991; Gallagher *et al.*, 2005; Serra-Pereira *et al.*, 2005a) and the most utilised function for other raijds (e.g. *D. oxyrinchus*, Yigin & Ismen, 2010; *R. clavata*, Serra-Pereira *et al.*, 2005b; Kadri *et al.*, 2014; the brown skate *Raja miraletus* L., 1758, Kadri *et al.*, 2012). According to Branstetter (1987), k values between 0.05 and 0.10 y^{-1} are typical of slow growing species, 0.10-0.20 y^{-1} of moderate growth animals and 0.20 - 0.50 y^{-1} of rapid growing species. Based on these categories, *R. brachyura* showed a relatively moderate growth rate, slightly more rapid in males ($k = 0.11$) than in females ($k = 0.10$). The ANCOVA test revealed statistical differences in age at length data between sexes confirming what was reported in Irish waters by Gallagher *et al.* (2005). The L_∞ values were also found to be quite similar between genders, with females reaching sizes slightly larger than males, as reported for the northeast Atlantic blonde ray (Holden, 1972; Gallagher *et al.*, 2005; Serra-Pereira *et al.*, 2005a). This trend is common among other batoids (e.g. Conwley, 1997; Licandeo *et al.*, 2006; Duman & Başusta, 2013; Fisher *et al.*, 2013). The maximum asymptotic lengths recorded in Sardinian waters were lower than those estimated in Irish (Holden, 1972; Fahy, 1991; Gallagher *et al.*, 2005) and Portuguese waters (Serra-Pereira *et al.*, 2005a) (Table 3). The low number of

Table 3. Length-weight relationships and von Bertalanffy growth parameters for *Raja brachyura* in different geographical regions.

Area	Sex	Number of fish (TL range, cm)	Total body mass and Total length (TM=a TL^b)			von Bertalanffy growth parameters			Method	References
			a	b	r^2	L_∞	k	t_0		
Atlantic Ocean										
Irish Sea	M	(Max 115)				115	0.19	-0.18	Tagging	Holden, 1972
	F	(Max 112)				118.4	0.19	-0.8		
Irish Sea	M	777				116.7-119.4	0.24-0.26	-0.31-+0.15	Vertebrae	Fahy, 1991
	F	910				120-144.3	0.19-0.29	-0.31-+0.15		
Irish Sea	M	127 (max 109)				145.8	0.145	-0.926	Vertebrae	Gallagher *et al.*, 2005
	F	141 (max 108)				154.7	0.129	-0.84		
British Isles	M	357 (13-100)	0.0027	3.256	0.99					McCully *et al.*, 2012
	F	386 (12-102)	0.0026	3.271	0.99					
English Channel	combined	100 (17-105)	0.0028	3.233	-					Dorel, 1986
Portugal	combined	139 (37-106)	-	-	-	133.5	0.12	0.29	Caudal thorns	Serra-Pereira *et al.*, 2005b
Portugal	combined	334 (38-106)	$1.19*10^{-6}$	3.20	-					Serra-Pereira *et al.*, 2010
Mediterranean Sea										
Asinara Island, Sardinia	M	123 (23-95.5)	$1.04*10^{-6}$	3.28	0.99					
(Central – Western Mediterranean)	F	102 (22.5-73.5)	$5.24*10^{-7}$	3.40	0.99					Catalano *et al.*, 2007
Sardinian waters (Central –Western Mediterranean)	M	862 (12-96.5)	0.0014	3.377	0.97					Present study
	F	930 (13.1-105.5)	0.0012	3.439	0.98					
	M	79 (18.4-95.5)				108.8	0.11	-1.2	Vertebrae	
	F	59 (25.2-90.8)				111.1	0.10	-1.3		

98

Medit. Mar. Sci., 16/1, 2015, 90-102

individuals bigger than 90 cm TL could have resulted in an underestimation of blonde ray L_∞ values in our samples. Furthermore, Atlantic studies noted higher k values, indicating a faster growth rate. As a result of the divergence in the growth parameters, we found a higher mean age than that observed in skates caught in the Atlantic Ocean. Indeed, the maximum observed age in Sardinian samples was 16 years (95.5 cm TL) versus an 8 year-old specimen (90-100 cm TL) reported by Gallagher *et al.* (2005) analysing the vertebral centra sections and an almost 10 year-old individual (~ 100 cm in TL) described by Holden (1972) through the tag and recapture method. This discrepancy could also be derived from other factors that may be affecting the growth of the species, such as environmental differences between the Mediterranean and Atlantic Ocean, the influence of water temperature, species behaviour, different exploitation state, etc.

The growth profile of all reproductive organs analysed by maturity stage, supported the different phases identified in major maturation processes of rajids (*e.g.* Ebert, 2005; Oddone & Vooren, 2005; Frisk & Miller, 2009).

Whereas most teleosts begin to mature between 40 and 80% of their maximum size (Beverton & Holt, 1959), elasmobranchs mature at a much larger relative size (Holden, 1972), with skates maturing at between 75 and 90% of their maximum TL (Ebert, 2005). In particular, in Sardinian seas, size at maturity was at 87.2 cm TL for females and 80.8 cm TL for males, corresponding to 83 and 83.7% of maximum observed length, respectively. These data, as reported by Ebert (2005), indicated that the blonde ray could be particularly sensitive to fishing pressure and overexploitation, a conclusion also supported by the late age at which females and males mature (14 and 10 years respectively).

Late age at maturity and with females maturing later than males, was previously observed in the Atlantic waters where similar sizes of L_{50} were obtained (Dorel, 1986; Gallagher *et al.*, 2005; McCully *et al.*, 2012; see Table 4). This maturity pattern in females could be associated with additional growth to attain larger abdominal cavity sizes as confirmed by the TL – DW relationships, which leads to increased fecundity and greater quality

and size of offspring (Serra-Pereira *et al.*, 2011). These finding were also confirmed by Gallagher *et al.* (2005) for Irish waters, where female and male sexual development starts at around 80 cm TL, which corresponds to the enlargement of the oviducal glands and clasper glands.

Within Sardinian waters, during the sampling period, all maturity stages were recorded. Immature females predominated during all the sampling seasons, in contrast to mature specimens. The latter occurred during a restricted period of the year from spring (late May) to summer with active females found mainly during summer. These results were also confirmed by the seasonal evolution of GSI values. Although a limited reproductive period was also found along the Portuguese coasts and British west coasts, timing coincidence among males and females was not occurring. Serra-Pereira *et al.* (2005a) indicated the presence of sexually active females only in March, while Clark (1922) and Holden *et al.* (1971) observed females with egg capsules from February to July. The intraspecific variations between Mediterranean and Atlantic specimens could be due to the geographic and hydrographic characteristics of the areas (Capapé, 1977).

We reported, for the first time, data on ovarian fecundity of *R. brachyura* in the wild, despite the low number of specimens analyzed. The blonde ray in Sardinian seas showed a very low fecundity ranging from 37 to 44 yolked follicles. This result appeared to be in agreement with the study conducted by Holden *et al.* (1971) in captivity in which the blonde ray laid a maximum of 0.5 egg per day (32 eggs in 77 days).

In conclusion, given the generalized stock collapse of skates from coastal ecosystems (Musick, 2004), and the lack of relevant legislation in the Mediterranean Sea, it may be pertinent to institute a minimum landing size (MLS) for rajids. However, a generic MLS for skates may not benefit all species as reported also by McCully *et al.* (2012). Indeed, the establishment of an effective MLS depends strictly on the life-history traits of each species. For this reason, the growth and reproductive parameters in the Mediterranean Sea provided for the first time in this study, represent the first step for future implementation of basic management measures aimed at ensuring

Table 4. Summary table for *Raja brachyura* estimates of length and age at maturity in different geographical regions.

Area	Sex	N	Length range (TL, cm)	First maturity (TL, cm)	Largest immature (TL, cm)	50% mature (L_{50}) (TL, cm)	50% mature (A_{50}) (age, years)	References
Atlantic Ocean								
English Channel	Combined	100	17-105	100	-	-	-	Dorel, 1986
Irish Sea	M	123	ca 15-103	75	-	81.9	4.63	Gallagher *et al.*, 2005
	F	61		81	-	83.6	5.50	
British Isles	M	359	13-100	55	91	78.2	-	McCully *et al.*, 2012
	F	389	12-102	60	93	85.6	-	
Mediterranean Sea								
Sardinian waters CW Mediterranean	M	930	12-96.5	74.6	80.0	80.8	10	Present study
	F	812	13.1-105.5	85	87.3	87.2	14	

Medit. Mar. Sci., 16/1, 2015, 90-102

99

the sustainability of catches for this species, given its relative abundance (Follesa *et al.*, 2010) and commercial importance in Sardinian seas.

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100

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101

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102

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