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Age, growth and length–weight relationship of the rough skate, *Raja radula* (Linnaeus, 1758) (Chondrichthyans: Rajidae), from the Gulf of Gabes (Tunisia, Central Mediterranean)

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PEER REVIEW

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Comments

This work is a good coastal medicine paper discussing on study and test of uncommon species in the area, the Gulf of Gabes. As noted, the result can be a good data for further worker in the field to assess and study the other rare species and stimulate report from this specific area.

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ABSTRACT

Objective: To investigate the length distribution, sex ratio, length–weight relationship, age and growth of *Raja radula* in the Gulf of Gabes.

Methods: The age and growth characteristic of the rough skate (*Raja radula*) inhabiting in the Gulf of Gabes (Central Mediterranean Sea) was determined. A total of 1250 specimens were sampled. According to the marginal increment band its growth was annual.

Results: The growth parameters were derived by using the von-Bertalanffy method and were separately evaluated as follows: $TL_{\infty}=97.2$ cm, $K=0.15$ y^{-1} , $t_0=0.35$, and $W_{\infty}=5.28$ kg, and $TL_{\infty}=76.4$ cm, $K=0.22$ y^{-1} , $t_0=0.16$, and $W_{\infty}=3.77$ kg, for females and males respectively. The maximum age was 12 years for females and 10 years for males.

Conclusions: Results from this research will provide a starting point to develop a management plan for the rough skate in the Gulf of Gabes. Further investigations are necessary to quantify the impact of the existing regulations on the population dynamics and recruitment patterns of this species in the region.

KEYWORDS

Age and growth, Mediterranean Sea, Gulf of Gabes, Vertebra, *Raja radula*

1. Introduction

Interest in skates is increasing because of their prevalence as a by-catch in groundfish fisheries throughout the world^[1–3]. The family Rajidae is considered to be one of the most vulnerable elasmobranch groups to overexploitation^[4,5].

The rough ray, *Raja radula* Delaroche 1809 (*R. radula*), is a relatively small skate endemic to the Mediterranean Sea^[6]. In spite of the increasing fishing pressure, there is a paucity of information on rough ray life history resulting in an assessment of 'Data Deficient' on International Union For Conservation Of Nature (IUCN) Red List assessments for Chondrichthyans in the Mediterranean Sea^[7].

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Off the Tunisian coasts, *R. radula* is frequently captured as a by-catch of demersal trawls throughout the year in shallow coastal waters[8].

There is very little information on the reproductive parameters of *R. radula*, and no studies on the age and growth of these species in the Mediterranean Sea such as the Gulf of Tunis[9], Gulf of Gabes[8], Cilician Basin shelf waters (Northeastern Mediterranean)[10], Ionian Sea[11]. This status in the Gulf of Gabes is unclear.

This study provides the first estimates of age and growth parameters of the rough skate in the Central Mediterranean (Tunisian south coast), based on vertebrae from wild-caught specimens collected in the Gulf of Gabes. The results derived from this study provide very important and useful information for conservation and management of this species.

2. Materials and methods

2.1. Sample collection

Fish samples were collected monthly from September 2007 to August 2009. Specimens were captured as by-catch at bottom trawler, longlines and demersal gill-nets 30–50 m depth in the Gulf of Gabes (Figure 1). In the laboratory a total of 1250 specimens were measured to the nearest centimeter for total length (TL), width disc and weighed to the nearest 10 g for total weight (W).

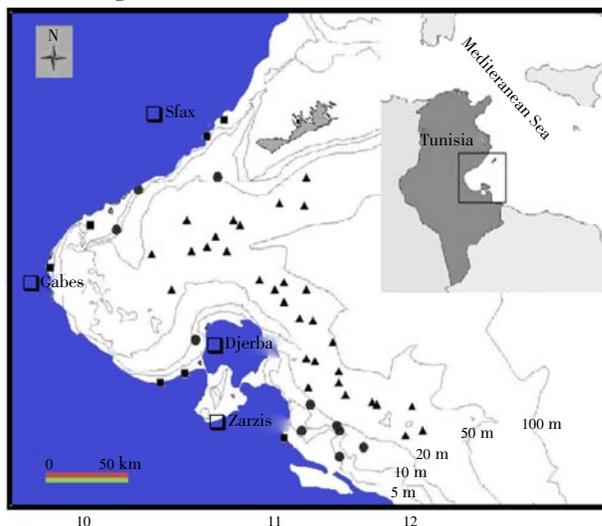


Figure 1. Map of the Gulf of Gabes (Tunisia, Central Mediterranean Sea). Triangle: Trawls; Squared: Demersal gillnets; circle: Longline.

2.2. Vertebrae preparation and age determination

Vertebral samples centres were taken from above the abdominal cavity of 165 *R. radula*, labeled, and stored frozen[12]. Soft tissue was removed from the frozen vertebral segments using a scalpel. The individual vertebrae were then cut apart from each other and soaked in warm distilled

water. Hypochlorite (5%) was used to remove the last remaining bits of connective tissue from the vertebrae. However, hypochlorite can decalcify cartilage when overused, so soak time was kept to nearly 10 min. The vertebrae were then air-dried for no less than 48 h. 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 paped to produce a thin section of 0.6 mm.

Vertebral sections were examined under a compound microscope and 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.[13]. Liquid clarifier (EDTA) was applied to the section surfaces to enhance the banding pattern. One growth band was defined as an opaque and translucent band pair that traversed the intermedia and clearly extended into the corpus calcareum[14].

The index of the average percentage error (IAPE)[15], the coefficient of variation (CV)[16], and the age-bias plot[17], were then used to compare reproducibility of the age determination between readings, as follows:

$$IAPE = \frac{1}{N} \sum_{j=1}^N \left(\frac{1}{R} \sum_{i=1}^R \frac{|X_{ij} - X_j|}{X_j} \right) \times 100$$

where *N* is the number of animals aged, *R* is the number of readings, *X_{ij}* is the count from the *j*th animal at the *i*th reading and *X_j* is the mean age of the *j*th animal from *i* readings.

$$CV = 100\% \times \frac{\sqrt{\frac{1}{R} \sum_{i=1}^R (X_{ij} - X_j)^2 / (R-1)}}{X_j}$$

Where CV is the age precision estimate for the *j*th fish; *X_{ij}* is the age determination of the *j*th fish by the *i*th reading; *X_j* is the mean age of the *j*th fish and *R* is the number of readings.

The von Bertalanffy growth equation (VBGE)[18] 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 *t₀*=the theoretical age at zero length.

The VBGE was calculated by using FISHPARM, a computer program for parameter estimation of nonlinear models with algorithm for least-square estimation of nonlinear parameters[19]. An analysis of covariance (ANCOVA) was used to compare the slopes between sexes.

2.3. Marginal increment analysis

The periodicity of band pair formation was investigated using the marginal increment ratio (MIR)[20]. A sub-sample

of 165 vertebrae were randomly selected comprising both juvenile and adult specimens collected in every month. The MIR was calculated as the ratio of the distance between the last and penultimate opaque bands as measured with an optical micrometer. The MIR was calculated by the following equation^[21]:

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

Where R is the centrum radius, R_n 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^[22].

The growth in mass was also described by the same model.

$$W_t = W_\infty (1 - e^{-K(t-t_0)^b})$$

Where W_t is the total mass at time t, W_∞ is the maximum theoretical mass of species and b is the power constant of the length mass relationships.

The relation of weight to length was calculated applying the exponential regression as the following equation:

$$W = a \times L^b$$

Where W is the total weight (g), L the total length (cm), b and a are parameters to be estimated. The regression lines between sexes ANCOVA statistic test was used.

The diameter of the first 10 vertebrae from each fish was measured and a mean vertebra diameter (V_d , ± 0.05 mm) was estimated for each individual. The relation between RV and TL was determined by regression analysis, using linear and non–linear models for a better comprehension of age and growth characteristics and the use of vertebrae in age estimates.

3. Results

3.1. Length frequency distribution

The sample was composed of 730 females and 520 males. The total length varied from 15 and 70 cm for males and from 15 and 80 cm for females (Table 1). The bulk of samples indicated that a peak occurred at a size of 45–65 cm TL for both sexes (Figure 2). The distribution of length within these ranges was significantly different (Kolmogorov–Smirnov test, $D=0.265$, $n=1\ 250$, $P=0.038$). Mean total length was greater for females than that for males.

Table 1

The mean \pm SD, minimum (Min), maximum (Max), and mode of total length (cm) for *R. radula* in the Gulf of Gabes.

| Parameter | Total length (cm) | | |
|---------------|-------------------|------------------|------------------|
| | All fish | Females | Males |
| Min | 15 | 15 | 15 |
| Max | 80 | 80 | 70 |
| Mean \pm SD | 58.92 \pm 0.07 | 56.22 \pm 0.18 | 42.35 \pm 0.16 |
| Mode | 51.2 | 55.1 | 48.3 |

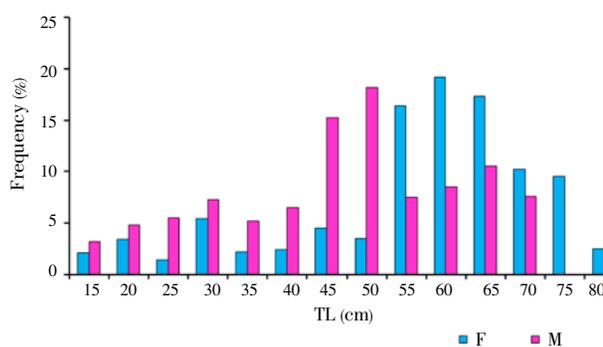


Figure 2. Length–frequency distribution of males and females *R. radula* caught in the Gulf of Gabes.

3.2. Length–weight and vertebra relationships

The length–weight relationships of both sexes separately and all fish are represented in Table 2. There was no statistically significant slope of the length–weight regressions between sexes (ANOVA, $P>0.05$).

Table 2

Length–weight relationships and regression parameters (a, b, r^2) of *R. radula* in the Gulf of Gabes.

| Sex | Equation | N | a | b | r^2 | Significance | Allometry |
|-----|---------------------|------|-------|------|-------|--------------|-----------|
| F | $W=0.011 TL^{3.35}$ | 520 | 0.011 | 3.35 | 0.98 | $P>0.05$ | positive |
| M | $W=0.017 TL^{3.32}$ | 730 | 0.017 | 3.32 | 0.96 | $P>0.05$ | positive |
| F+M | $W=0.014 TL^{3.34}$ | 1250 | 0.014 | 3.34 | 0.97 | $P>0.05$ | positive |

The b value was significantly higher than the theoretical value of 3 for females (t –test, $t=1.84$, $P<0.05$), for males (t –test, $t=2.18$, $P<0.05$) and for all individuals (t –test, $t=1.59$, $P<0.05$) which means a positive allometric growth pattern ($P<0.05$).

Consequently, the different classes of age of *R. radula* were subdivided among age groups. The length–age key of all individuals by age class and length class is presented in Table 3. The different classes of age of rough skate were subdivided into age groups. Fish aged 1 to 12 years old were recorded (Table 3). During the first year of life, fish attained over 8% of their maximum observed length. By the end of the 7 and the 12 years, specimens attained approximately the 85% and the 92% of their maximum length, respectively. Indeed, over 80% of the aged specimens were between 10 and 12 years. The length–age key of all individuals by age class and length class is presented in Table 3.

Given a strong linear correlation between the total length and vertebra radius (Table 4), vertebra radius measurements were used to back calculate the total length of previous ages.

3.3. Marginal increment ratio

When the sexes were combined, marginal increments were significantly different between months (Kruskal–Wallis test, ($H=18.15$, $df=6$, $P=0.04$) and was at its minimum during the months of January and February. Furthermore,

monthly categorization of vertebral edges indicated that the highest frequency of opaque edges appears during January suggesting that a single opaque band may be formed annually on vertebral centra during this period (Figure 3).

Table 3

Age-length key of 165 individuals of *R. radula* from the Gulf of Gabes. CI, confidence intervals.

| LT (cm) | Sex | Age (year) | | | | | | | | | | | | |
|-----------------|-----|------------|------|------|------|------|------|------|------|------|------|------|------|------|
| | | 0+ | 1+ | 2+ | 3+ | 4+ | 5+ | 6+ | 7+ | 8+ | 9+ | 10+ | 11+ | 12+ |
| 16.5-18.4 | M | 1 | | | | | | | | | | | | |
| | F | 1 | | | | | | | | | | | | |
| 18.5-29.9 | M | | 2 | | | | | | | | | | | |
| | F | | 2 | | | | | | | | | | | |
| 30.0-33.9 | M | | 1 | | | | | | | | | | | |
| | F | | | | | | | | | | | | | |
| 34.0-37.9 | M | | | 3 | | | | | | | | | | |
| | F | | | 2 | | | | | | | | | | |
| 38.0-41.9 | M | | | 3 | | | | | | | | | | |
| | F | | | 3 | | | | | | | | | | |
| 42.0-45.9 | M | | | | 2 | 3 | | | | | | | | |
| | F | | | | 2 | 4 | | | | | | | | |
| 46.0-49.9 | M | | | | 1 | 3 | 3 | | | | | | | |
| | F | | | | 1 | 2 | 4 | | | | | | | |
| 50.0-53.9 | M | | | | | 2 | 5 | 3 | | | | | | |
| | F | | | | | 2 | 5 | 4 | | | | | | |
| 54.0-57.9 | M | | | | | | | 8 | 3 | | | | | |
| | F | | | | | | | 6 | 2 | | | | | |
| 58.0-61.9 | M | | | | | | | | 3 | 6 | | | | |
| | F | | | | | | | | 4 | 6 | | | | |
| 62.0-65.9 | M | | | | | | | | | 4 | | | | |
| | F | | | | | | | | | 5 | | | | |
| 66.0-69.9 | M | | | | | | | | | | | | | |
| | F | | | | | | | | | 7 | 5 | | | |
| 70.0-73.9 | M | | | | | | | | | | | | | |
| | F | | | | | | | | | 9 | 3 | 6 | | |
| 74-78 | M | | | | | | | | | | | | | |
| | F | | | | | | | | | 4 | 6 | 9 | | |
| Total | | 2 | 5 | 11 | 6 | 16 | 17 | 21 | 12 | 41 | 14 | | | |
| Moyenne LT (cm) | | 16.8 | 26.6 | 37.2 | 44.6 | 51.2 | 56.9 | 61.6 | 65.9 | 68.8 | 74.9 | 78 | 80.6 | 80.8 |
| CI (±) | | 2.2 | 3.42 | 1.14 | 2.23 | 1.45 | 2.04 | 1.35 | 4.63 | 6.42 | 2.24 | 1.22 | 1.08 | 1.35 |

CI: Confidence intervals.

Table 4

Relationships between total length and vertebra radius of *R. radula* from the Gulf of Gabes.

| Sex | No. | a | b | r ² | Size range (cm) |
|--------------|-----|--------|------|----------------|-----------------|
| Combined sex | 165 | 229.70 | 6.20 | 0.92 | 15-80 |
| Females | 85 | 237.67 | 7.40 | 0.93 | 15-80 |
| Males | 80 | 221.25 | 5.08 | 0.92 | 15-70 |

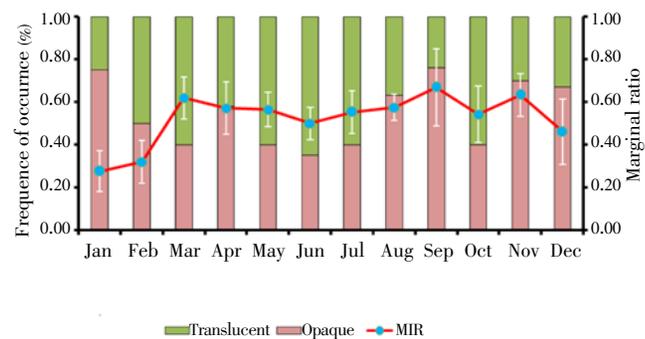


Figure 3. Monthly changes in the marginal increment (MIR, mean±SE) for *R. radula* (combined sexes), from the Gulf of Gabes.

3.4. Age and growth

The remaining 165 vertebrae, 85 females (15-80 cm TL) and 80 males (15-70 cm TL) were used for age estimation. After separate readings, exact agreement of ring counts were reached on 5% differed by one ring, 3% by two rings and 3% by more than three. The average IAPE of the overall sample was 3.24% and the CV was 2.15. The levels of precision indicated a high level of reproducibility, so these data were used for further analyses.

For the calculation of the fish total length and vertebra radius relationship the data from 165 individuals are presented in Table 4 for both sexes and combined sexes. No significant difference was found between the slopes of females and males (ANCOVA, $P>0.05$) (Table 4).

The oldest males were estimated to be 10 years old (70 cm TL), and the oldest females to be 12 years old (80 cm TL) (Figure 4). Estimated parameters of von Bertalanffy growth

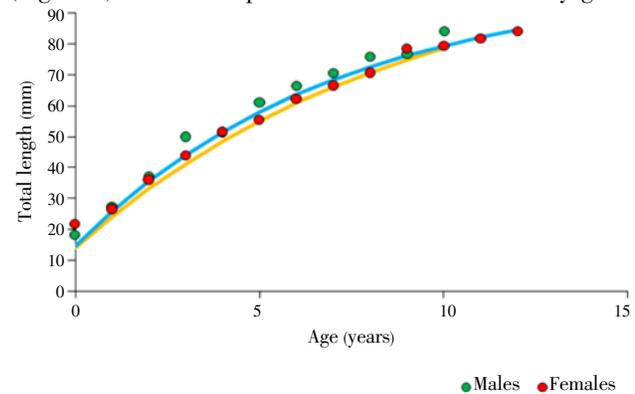


Figure 4. Von Bertalanffy growth curve for females and males of *R. radula*, from the Gulf of Gabes.

Table 5

Estimates of von Bertalanffy growth parameters TL_{∞} , W_{∞} , k and t_0 for females, males, and combined sex of *R. radula* in the Gulf of Gabes.

| Sex | TL_{∞} (cm) | W_{∞} (kg) | K (year ⁻¹) | t_0 (year) |
|--------------|--------------------|-------------------|---------------------------|--------------|
| Females | 97.20±1.40 | 5.28 | 0.15±1.03 | 0.35±0.01 |
| Males | 76.40±1.31 | 3.77 | 0.22±0.09 | 0.16±0.04 |
| Combined sex | 86.40±1.27 | 4.10 | 0.19±0.02 | 0.25±0.04 |

3.5. Age at first maturity

Based on observations of 550 females and 400 males, the 50% TL was estimated to be 47.05 cm for males and 56.48 cm for females. The age at 50% of mature was estimated to be 4.47 years for males and 5.89 years for females[8].

4. Discussion

The amount of research on *R. radula* is rather limited and there is no known study on the age determination in this species. This is the first known research made on age reading of *R. radula*. The average length values of these rays were between 56.22 and 42.35 cm. The longest male and female measured 70 and 80 cm in TL, respectively.

From the length–weight relationship values; the “b” value is 3.34 for both sexes, 3.35 for females and 3.32 for males. According to the “b” values obtained for both sexes, the female of the species showed a isometric growth characteristic. Males showed negative allometric growth. Although it is determined differences between growth characteristic of female and males, no differences in slope were found between sexes ($P < 0.05$), indicating that weight increased allometrically with length.

Females were larger and heavier than the males. Similar patterns were observed for *Raja miraletus*[23], *R. radula* and *Raja clavata* from off Tunisian coast[8].

The relationship between animal length and vertebral radius suggested that *R. radula* vertebrae continued to grow throughout life, making them a suitable structure for estimating age and investigating growth. In fact, these structures were used frequently for age determination of *R. radula*. The direct relationship between vertebrae and fish parameters suggests that the vertebrae radius pattern reflects that of the fish since they are both controlled by the same metabolic processes[8].

Vertebrae radius of *R. radula* show the common ring pattern of temperate fishes with one opaque mark and one translucent mark formed each year corresponding to rapid and to slow growth, respectively.

There are various approaches for verifying age estimation methods of which edge analysis and MIR are among the most frequently employed. In this study MIR was calculated. These methods focus on incremental patterns of growth–band pairs throughout the year. The annual periodicity in band pair formation observed in the Gulf of Gabes is supported by other studies[8,23]. According to the MIR it was found that age band growth was annual. Assessment and management of marine fisheries can be negatively affected by misspecification of ages[24].

The values of the IAPE and the CV suggested that the precision levels obtained are according to the reference point values indicated by Campana[25].

Values of TL_{∞} determined in our work revealed that adult females grew larger than did adult males, whereas the value of K for males was higher than that for females. Male growth rates tended to be faster, which is in agreement with the results of previous studies for other *Rajid* species[7,26–28].

In our study, the age and growth parameters for the rough skate showed that *R. radula*, as well as other elasmobranchs, requires conservative management because of its slow growth rate and vulnerability to over–exploitation[20,29,30].

The growth pattern of *R. radula* population of the Gulf of Gabes presented in this paper provides several key biological parameters. The maximal lengths of males and females in this study were 70 and 80 cm, respectively. The maximum age encountered in our samples was estimated 12 years and 10 years for females and males respectively.

Results from this research will provide a starting point to develop a management plan for the rough skate in the Gulf of Gabes. Further investigations are necessary to quantify the impact of the existing regulations on the population dynamics and recruitment patterns of this species in the region. The data from this study can be used for stock assessment investigations in future.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

This is a diagnostic test to study a local rarely mentioned species from an area with limited data, gulf area. In short, this work is very interesting and should be cited in the further study.

Research frontiers

Some interesting new data from field work on the local species in Gulf area can be seen in this work. It can be a good report in the field and can trigger the follower to perform addition studies. Future relating citation can be expected.

Related reports

There are some limited reports from this area. Therefore, this work shows some new data and insight from the field, Gulf area.

Innovations and breakthroughs

Although there is no new intervention some new information on rarely mentioned species in the area with limited research can be derived from this study. The new insight in the field can be a useful point emerged from this article. The work can be a good example for other researchers to follow and cite.

Applications

This work can be applied in the field of coastal medicine. The result can be a good data for further research and

verification. Additional extension study to cover other rare species in the Gulf area can be expected. Further study on this area can be expected.

Peer review

This work is a good coastal medicine paper discussing on study and test of uncommon species in the area, the Gulf of Gabes. As noted, the result can be a good data for further worker in the field to assess and study the other rare species and stimulate report from this specific area.

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