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First Estimates of Age and Growth of the Lusitanian Cownose Ray (*Rhinoptera marginata*) from the Mediterranean Sea

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Abstract: Within the eastern Mediterranean, increased fishing pressure coupled with a lack of basic biological information is threatening the sustainability of the rare Lusitanian cownose ray (*Rhinoptera marginata*). To provide baseline life-history data for this species, age estimates were obtained from vertebral band counts of 224 rays (size range: 210–998 mm disc width and 140 to 13,600 g weight) collected from Iskenderun Bay. Females ranged in size from 210 to 998 mm disc width and 238 to 13,600 g weight, while males ranged from 130 to 866 mm disc width and 140 to 8250 g weight. The index of average percent error (2.8%) and age-bias plot suggest that the aging method used represents a precise and non-biased approach. Marginal increment analysis indicated that a single opaque band is deposited annually between August and September. The oldest ages obtained for *R. marginata* were 9 years for males and 19 years for females, which corresponded to total lengths of 866 and 998 mm disc width, respectively. For males, limited samples prevented the accurate calculation of growth rates; however, for combined sexes, observed and disc width-at-age data resulted in the following von Bertalanffy growth parameters: DW_∞ = 1102.16 mm, k = 0.148 and t_0 = −0.2167. Although additional samples are necessary to determine growth rates in males, the results of the present study indicate that *R. marginata* females exhibit life history characteristics similar to other *Rhinoptera* species.

Keywords: elasmobranch; Rhinopteridae; batoids; age; growth; Mediterranean

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1. Introduction

The Lusitanian cownose ray (*Rhinoptera marginata*, Geoffroy Saint-Hilaire, 1817) is a large epibenthic ray that inhabits sandy or muddy substrates on the continental shelf (depth range: 20 to 100 m) along the west coast of Africa and the Mediterranean Sea [1,2]. Although commonly captured in various African fisheries due to its abundance inshore, the Lusitanian cownose ray is considered rare within Mediterranean waters and is the only member of *Rhinoptera* spp. reported to inhabit this region [3,4]. This species is listed as "Data Deficient" in this region and globally assessed as "Near Threatened" by the IUCN [5]. Due to their rare occurrence and recent increases in both direct and indirect fishing pressure, the sustainability of this ray, along with other elasmobranchs, is threatened within the Mediterranean. Unfortunately, high at-sea discard rates (driven by low market values for sharks and rays) and unregulated harvests have significantly limited the collection of biological data pertaining to this and other cartilaginous fishes in this region [6–8]. For example, data regarding the Lusitanian cownose ray is limited to length–weight relationships, distribution studies and a brief reproductive investigation [2,9–12]. Very little is known about any migratory behaviour of the Lusitanian cownose ray in the Mediterranean Sea. However, we know that the most studied *Rhinoptera* species, *R. bonasus*, displays migratory behaviour in its native geographical distribution. Our guess is that the Lusitanian cownose ray is distributed across a very wide range in the Mediterranean Sea. In an earlier study, Tirasin and Basusta [13] found a total of 129 individual rays within a school formation very close to the present study area. Of the 129 specimens, 89 were female and 40 were male. When dissected, 36 female fish were found to be gravid, each bearing one near-term embryo. The authors, after presenting a great deal of information and discussing the findings from other relevant publications, concluded that the incidental catch of such a large number of fish, including many gravid specimens with near-term embryos and mature males together in one single haul, suggested that the rays were in a schooling formation when they were captured. The males may have been following the females so that they could maximise their chances of mating with them soon after parturition. They also argued that the location of the haul in Mersin Bay—a marine area in the vicinity of the estuaries of two rivers, the Tarsus and Seyhan—and its relative closeness to the sampling location of the neonate in Iskenderun Bay prompt a hypothesis that these fish may be using this region as a reproduction and nursery area. The Yumurtalık Bight and the estuaries of big rivers such as the Seyhan and Ceyhan are likely candidates for a Lusitanian cownose ray nursery-ground in the northeastern Mediterranean. There have also been previous observations of neonates, small juveniles and gravid females (N. Başusta, pers. obs.) in this region, which provide additional support for this proposition. Without a more comprehensive understanding of this species' life history, managers cannot accurately assess population levels and establish protective regulations to prevent its future exploitation [14].

Age information is an important variable to understand the population condition since it provides basic information needed to determine growth and mortality rates. Furthermore, a lack of age and growth information impedes the development of adequate fishery management regulations [15–17]. In addition, it is well-established that a lack of age and growth information is a limiting factor in the development of chondrichthyan management plans [18]. Given this, the objective of the present study was to establish age and growth rates for the Lusitanian cownose ray based on vertebral band counts from specimens collected in Iskenderun Bay, Turkey.

2. Materials and Methods

Lusitanian cownose rays were collected between September 2010 and February 2012 by commercial purse-seine and bottom-trawl fisherman in the coastal waters (depth range: 25–50 m) of Iskenderun Bay (Figure 1). In the laboratory, each ray was thawed and its sex and external morphological measurements, including disc width (DW; mm; measured as a straight line distance between the wing tips of the widest portion of the pectoral fins using a metric tape) and weight (W; measured to the nearest g using a digital balance), were recorded. Sex was macroscopically determined by the presence (male) or absence (female) of claspers. Age estimates were determined from gross vertebral band counts, processed following the protocol described by Sulikowski et al. [19]. Briefly, three individual centra were soaked in a sodium hypochlorite (6%) solution (<10 min to prevent decalcification) to remove any remaining tissue, then rinsed in distilled water and air dried for 48 h [15,20]. A sagittal cross-section was then cut from each centrum using a Raytech® gem saw and subsequently affixed to a microscope slide. If the banding pattern was not immediately apparent, the mounted cross-sections were sanded with fine-grit wet-dry sandpapers until clear bands were observed. Cross-sections were photographed using a Leica S8APO brand microscope with Leica Application Suit (Ver. 4.8.0) software (Figure 2). Two non-consecutive band counts were made by two independent readers for each specimen without prior knowledge of the specimens' DWs or previous counts. If band counts differed by more than 2 years, the specimen was removed from further analysis. Count reproducibility was calculated using the index of the average percentage error (IAPE) and an age-bias plot was used to evaluate age-bias between readers [15,21,22].

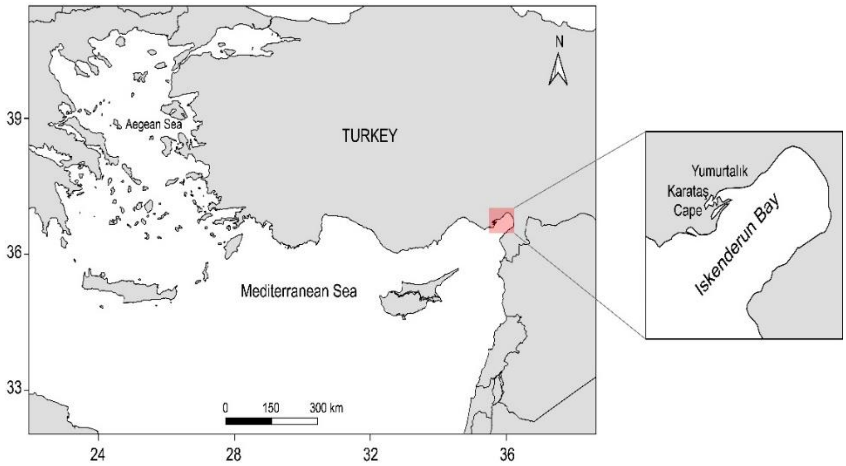

Figure 1. The triangle indicates Iskenderun Bay, Turkey, where the *Rhinoptera marginata* specimens were collected.

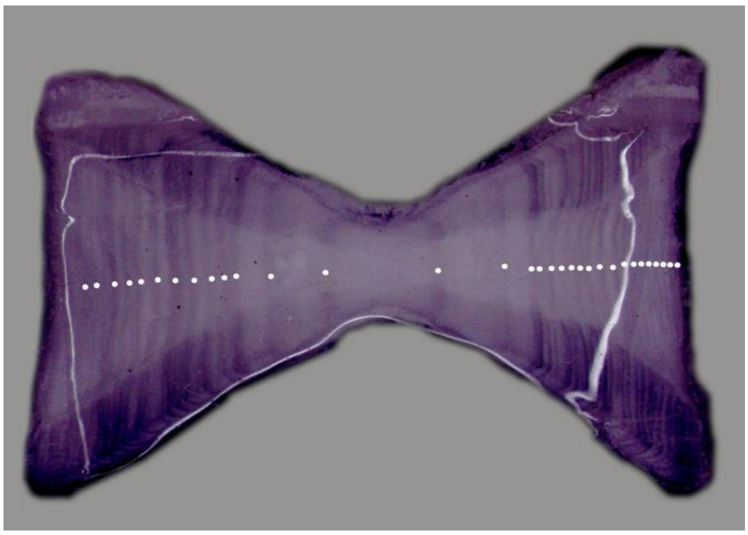

Figure 2. Longitudinal cross-section of a vertebral centrum from a 982 mm disc-width female *Rhinoptera marginata* estimated to be 18 years old. White dots denote opaque bands.

The annual periodicity of band pair formation was verified using the marginal increment ratio (MIR) [19] based on 45 specimens (juvenile and adults) collected across all months with the exception of June, July and December. The MIR was calculated as the ratio of the distance between the last and penultimate opaque bands as measured using Digimizer image analysis software (Ver. 4.8.0). The average MIR was plotted by month of capture to identify trends in band formation, and Kruskal–Wallis one-way analysis of variance on ranks was used to test for differences in the marginal increment by month [19,23].

Due to the opportunistic collection method employed in this study, insufficient samples were obtained for the smallest size classes for females. As a result, for females the DWs at previous ages were back-calculated from centra measurements using the linear-modified Dahl–Lea method:

$$DWi = DWc\ [(a + bCRi)/(a + bCRc)] \quad (1)$$

where a and b are the linear fit parameter estimates, DWi is the length at ring i, DWc is the length at capture, CRc is the centrum radius at capture and CRi is the centrum radius at ring i [24]. For males, samples for both small and large (i.e., individuals with DWs > 866 cm) size classes were insufficient, limiting the ability to fit growth curves (even if back-calculations were employed) that would accurately reflect the population. As such, growth analysis was not performed for males and only direct age estimates are reported.

Growth parameters for combined sexes were then determined by fitting observed DW-at-age data and DW-at-age data with and without back-calculations using the von Bertalanffy growth function (VBGF) [25]:

$$DW_t = DW_\infty \left[1 - e^{-k(t-t_0)}\right] \quad (2)$$

where DW_t is the expected disc width at age t years, DW_∞ is the asymptotic average maximum disc width, k is the growth coefficient and t_0 is the theoretical age at zero length. The data were also fitted with a Gompertz growth model [26,27], which is an S-shaped double exponential growth model, using the equation:

$$DW_t = DW_\infty \left[e^{-e^{(k(t-t_0))}}\right] \quad (3)$$

where DW_t is the mean disc width at time t, DW_∞ is the theoretical asymptotic length, k is the growth coefficient and t_0 is the theoretical age at zero length.

3. Results

3.1. Size Composition and Vertebral Analysis

In total, 224 (170 females and 54 males) Lusitanian cownose rays were collected and their ages assessed. Females ranged in size from 210 to 998 mm DW and 238 to 13,600 g W, while males ranged from 130 to 866 mm DW and 140 to 8250 g W (Table 1). Vertebral samples from all specimens (100%) were readable and the age assessments from both readers were within two years, resulting in an IAPE of 2.85%. Moreover, the comparisons of these counts indicated that no appreciable bias existed between readers (Figure 3). Captured males ranged in age from 0 to 9 years, while females ranged in age from 1 to 19 years.

Table 1. Mean disc widths (DW) and weights (W) at different ages for *Rhinoptera marginata* captured in the northeastern Mediterranean Sea. Values are given as means ± SD; sample sizes are given in parentheses.

Age (Years)	Female DW (cm)	Female W (g)	Male DW (cm)	Male W (g)
0	-	-	13.0 (1)	172
1	21.4 ± 5 (2)	270 ± 45	22.7 (2)	140
2	32.0 (1)	328	33.0 (1)	398
3	39.6 (1)	642	37.5 (1)	536
4	45.5 (1)	1290	-	-
5	55.6 ± 32 (8)	1895 ± 354	58.0 ± 22 (10)	2381 ± 309
6	64.4 ± 27 (21)	3222 ± 682	63.5 ± 23 (12)	3159 ± 806
7	72.1 ± 25 (30)	4836 ± 761	71.9 ± 21 (16)	4093 ± 807
8	79.5 ± 27 (52)	7301 ± 1445	79.5 ± 22 (11)	6266 ± 1240
9	85.4 ± 6 (14)	8919 ± 882	86.6 (1)	7706
10	87.4 ± 9 (10)	9630 ± 2163	-	-
11	89.7 ± 4 (9)	10,712 ± 849	-	-
12	91.3 ± 6 (8)	10,668 ± 1185	-	-
13	92.3 ± 1 (3)	10,520 ± 1091	-	-
14	93.9 ± 4 (3)	12,170 ± 1517	-	-
15	95.9 (1)	11,586	-	-
16	96.9 (1)	12,672	-	-
17	97.5 ± 6 (3)	13,433 ± 175	-	-
18	99.3 (1)	13,100	-	-
19	99.8 (1)	13,260	-	-

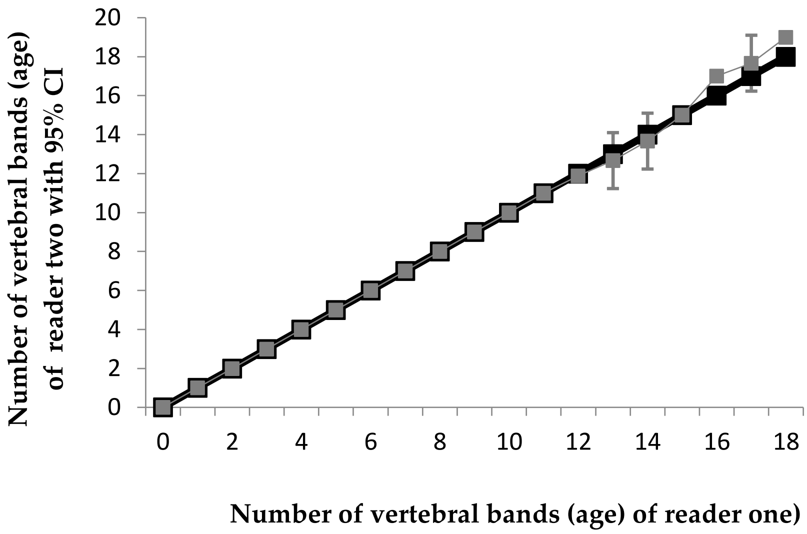

Figure 3. Age-bias plot of pairwise comparison of 224 *Rhinoptera marginata* vertebral counts made by two independent readers. Error bars represent the 95% CIs for the mean age assigned by reader two (grey line) to all specimens assigned a given age by reader one. The solid black line represents the one-to-one equivalence line. Sample sizes are given for each corresponding age.

Disc widths (DW) and weights (W) were compared between females and males aged 5–8 years using the Kruskal–Wallis test (KWT) as the data did not show a normal distribution. Firstly, before starting the analysis of the data, the distribution of the data was examined with the Kolmogorov–Smirnov test. Since $p < 0.05$ here, the data did not show a normal distribution. There was a significant difference between disc width and weight. The mean rank of females was higher than that of males.

3.2. Marginal Increment Analysis

Marginal increment widths were significantly different between all consecutive months (Kruskal–Wallis $p < 0.001$), with increment growth beginning in January and peaking in August (Figure 4). This peak was followed by a large decline in marginal increment growth, reaching minimum values in September. As such, these data suggest that a single opaque band is formed annually on the vertebral centra between August and September.

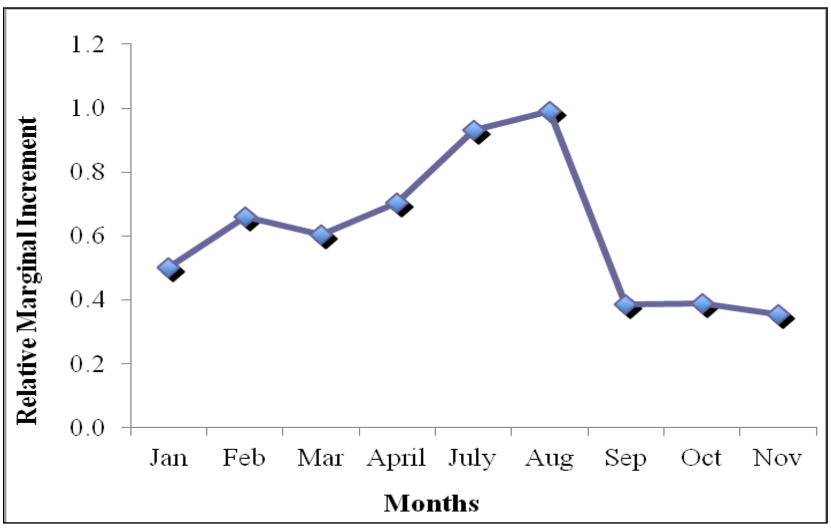

Figure 4. Mean monthly marginal increments of opaque bands for *Rhinoptera marginata* from the northeastern Mediterranean.

3.3. Back Calculation and Growth Estimates

The mean size at birth for females was estimated to be 177.9 mm using back-calculation. The back-calculated DWs were on average more than 63% greater than the observed DW-at-age for each corresponding age class (Table 2). A three-parameter von Bertalanffy growth curve was fitted to the combined observed and back-calculated age data for females only (Figure 5). The resulting model provided a good fit ($r^2 = 0.99$) to DW-at-age data and produced the following growth coefficients: $DW_\infty = 178.6$ cm, $k = 0.122$ and $t_0 = -2.082$. However, for combined sexes, $DW_\infty = 1102.16$ mm, $k = 0.148$ and $t_0 = -0.2167$.

Table 2. Back-calculated mean disc width (DW; cm) and observed mean disc width (cm) at band formation for female *Rhinoptera marginata*.

Band Number	0	1	2	3	4	5
Back-calculated DW	17.8	45.1	51.7	57.1	61.6	
S.D.						
N						
Observed DW		21.4	32.0	39.6	45.5	55.6
S.D.		5				32
n		2	1	1	1	8

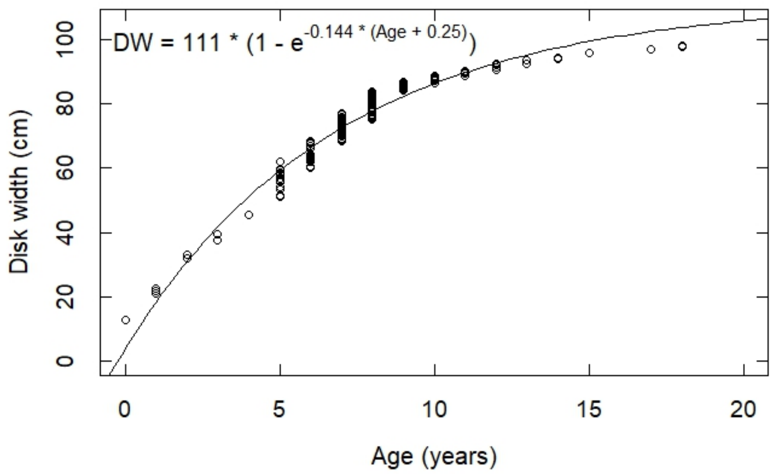

Figure 5. Generated von Bertalanffy growth curve for female *Rhinoptera marginata*.

The combined-sexes Gompertz model predicted a DW_∞ of 101.01 cm DW, a k value of 0.26935 per year and a t_0 of 2.88789 years (Figure 6).

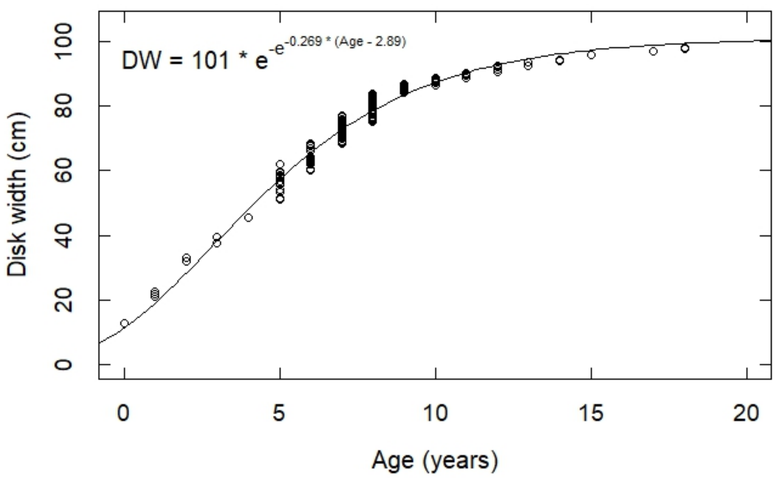

Figure 6. Generated Gompertz growth curve for female *Rhinoptera marginata*.

4. Discussion

Increases in fishing pressure coupled with unregulated harvests and incomplete biological profiles are several of the factors currently influencing the decline of Mediterranean batoid populations [2,28,29]. To avoid further exploitation of these chondrichthyan fishes and promote sustainability, the collection of accurate life history data is imperative [30]. The present findings clearly indicate that, like many other ray species, Lusitanian cownose rays are also slow-growing fish with a long life span. In addition, their delayed age of maturation and very low fecundity [13] make this species extremely susceptible to exploitation by fisheries, and therefore the species is in urgent need of conservation and protection. This study is the first to describe the age and growth of the Lusitanian cownose ray *Rhinoptera marginata* in the Mediterranean Sea.

A strong linear relation between the centrum diameter and DW indicates that the vertebra provide a continuous record of body growth and are a suitable structure for age interpretation in both sexes. Furthermore, the low (i.e., 2.85%) precision estimate coupled with the age bias suggests that our growth band interpretation represents a precise and non-biased approach for aging this ray. Marginal increment analysis verified that a single band pair is formed annually, with an opaque band being deposited during the fall (September–November). Although previous studies could not verify the month of band deposition, these results compare favourably to annual vertebral banding patterns reported in *R. bonanus*, a closely related cownose ray, found in the northwestern Atlantic and the Gulf of Mexico [31,32], as well as other similar sized batoids [19].

Among the eight globally recognized *Rhinoptera* species, age and growth data have only been previously reported for *R. bonasus* captured in the northwestern Atlantic (i.e., Chesapeake Bay) and the Gulf of Mexico (Table 3; [31–34]). Collectively, these studies suggest *R. bonasus* is sexually dimorphic, with females reaching larger maximum sizes than males. Although the observed size distribution of Lusitanian cownose rays reported in the present study potentially supports this pattern of sex-specific growth differences, the observed maximum sizes in both males and females were consistently smaller in comparison to *R. bonasus* (Table 3). Furthermore, Ebert and Stehmann [35] reported that the maximum size for Lusitanian cownose rays ranges from 1500 to 2000 mm DW, suggesting that the largest, and presumably oldest, individuals (both males and females) were likely not assessed in the present study. As such, it remains unclear whether the smaller observed maximum size in males (i.e., 866 mm DW) is a potential indication that this species is also sexually dimorphic or an artefact of the opportunistic collection method utilized in the present study. However, when the arithmetic averages are examined, it can be seen that the average for females is higher than that of males. Therefore, it is possible to confirm the existence of sexual dimorphism in this species.

The largest Lusitanian cownose ray we encountered during the study was a 998 mm DW female, close to the DW_∞ of 101.01 cm predicted by the Gompertz growth model. According to Neer and Cailliet [36], the Gompertz growth model provides the best fit for the size-at-age data for *Torpedo californica*. Mollet et al. [37] found that the Gompertz growth model best described the growth of *Dasyatis violacea*, indicating that it provided more realistic estimates of the growth rate than the VBGF.

Table 3. Comparison of von Bertalanffy-derived DW_∞, observed maximum DW and age for various *Rhinoptera* species.

Species	Sex	n	Observed Max DW (cm)	Observed Max Age (Years)	DW_∞ (cm)	t_0 (Years)	k	Locality	Reference
R. bonasus	F	40	107	13	125	−3.764	0.119	Chesapeake Bay (USA)	Smith and Merriner [31]
	M	51	98.1	8	119.2	−3.699	0.126		
R. bonasus	F	121	102.5	18+	123.8 *	−5.4799 *	0.0746	Northern Gulf of Mexico	Neer and Thompson [32]
	M	106	96	16+					
R. bonasus	F	260	110.5	21	106.3	−2.64	0.1931	Chesapeake Bay (USA)	Fisher et al. [33]
	M	140	98	18	97.1	−2.14	0.2741		
R. marginata	F	170	99.8	19	178.6	−2.082	0.122	Iskenderun Bay (Turkey)	Present study
	M	54	86.6	9	na	na	na		

* von Bertalanffy growth parameters were not significantly different between sexes.

For female Lusitanian cownose rays, the asymptotic disc width (DW_∞; 1786 mm DW) estimated with the VBGF was significantly greater than the observed maximum sizes (998 mm DW). Similar size discrepancies were also reported for *R. bonasus* [31] captured in Chesapeake Bay, ML, USA, which the authors attributed to small sample sizes (i.e., for females, n > 50) and the inclusion of only a few older, larger individuals (i.e., one animal > 10 years). It is well-documented that VBGF parameters can be altered if the available size-at-age data do not accurately reflect the population [14]. In the present study, only seven females with DWs > 939 mm (and ages >15 years) were collected. The lack of older individuals and larger size classes could have prevented the growth curves from reaching an asymptote, resulting in DW_∞ calculations that overestimated the maximum size [33]. In contrast, calculated growth rates for female Lusitanian cownose were comparable to those reported for *R. bonasus* captured in Chesapeake Bay (k = 0.119 [31]; 0.122 [33]) and other similar-size batoids [19,38,39]. Collectively, these data are in agreement with the assumption that larger batoids (i.e., bat rays (*Myliobatis californica*)—DW_∞ = 1587 mm; k = 0.099; oldest female = 23 years [38]; thorny skates (*Raja clavata*)—DW_∞ = 1200 mm; k = 0.13; oldest female = 16 years [19]; barndoor skates (*Dipturus laevis*)—DW_∞ = 1550 mm; k = 0.10; oldest female = 11 years [39]) are longer lived and slower growing than small species such as the round stingray (*Urotrygon ogersi*), which is a practically fast-growing species (DW_∞ = 208 mm; k = 0.22; oldest female = 8 years) [40].

5. Conclusions

This study found that Lusitanian cownose rays exhibit life history characteristics similar to other *Rhinoptera* species and are long-lived and slow growing, which are characteristics that have made other ray species vulnerable to overexploitation by commercial fisheries. Owing to new market interest in this species, filling in life-history gaps such as growth rates in males is essential.

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