Assessing thornback ray growth pattern in different areas of Western-Central Mediterranean Sea through a Multi-Model Inference analysis

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ABSTRACT

The lack of information on age validation often affects ageing studies in cartilaginous fish, even in the most common species. Recently, the annual growth band deposition pattern has been directly validated for thornback ray in the Mediterranean Basin, thus providing a highly reliable protocol for age reading. In this regard, taking advantage of this new information, the present study aims to investigate this species growth for the first time over a wide area of the Western and Central Mediterranean Sea, involving four different FAO geographic sub-areas. In order to provide an accurate description of the species growth, different models were fitted to the age-length data obtained from the observation of vertebral *centra* thin sections. A total of 720 specimens were analysed (358 females and 362 males) with total length ranging between 11.5 and 86.4 cm. The corrected Akaike's Information criterion (AICc) indicated, among the tested models, the three-parameter von Bertalanffy function as the most robust in describing the species growth. Growth modelling outcomes revealed the thornback rays as capable of growing relatively fast during the first years; the growth rate gradually slows down allowing maximum sizes of about 90–100 cm in total length to be reached. Significant discrepancies in the growth pattern were found between sexes in all investigated areas with the only exception being in the Northern Tyrrhenian Sea. Differences in growth patterns were observed between areas, specifically in the estimated growth rate of the species for each sub-region. The present study, confirmed the importance of the availability of a validated ageing protocol and testing multiple growth models. Moreover, our results highlighted the urgent need to investigate a species growth in a wide geographic area, searching also for possible differences at sub-region level. Providing this information could indeed allow eventual management plans to be adapted to the exact growth pattern that the species exhibit in the region, in this way increasing their effectiveness.

1. Introduction

Cartilaginous fish are globally indicated as one of the most highly threatened vertebrate groups (Dulvy et al., 2014) due to their particular life history traits which are typical of k-selected life strategy species (Cortés et al., 2012). This peculiar condition renders these animals highly susceptible to anthropogenic disturbances and, in particular, to fishing exploitation (Ferretti et al., 2010; Dulvy et al., 2014). These species are commonly found as a by catch in fisheries worldwide (White et al., 2012; Ellis et al., 2016), in this regard, the global increase of fishing pressure appears to put cartilaginous fish under an even higher risk, imposing the need for effective conservation and management measures (Dulvy et al., 2014).

Nonetheless, these kind of actions require a solid assessment of population status, which, besides the availability of reliable time series of catch records (Ligas et al., 2013) must be based on reliable

information regarding species life history traits. Among them, the description of growth pattern has always been considered as crucial since age and growth data are essential to obtain mortality data and productivity estimation (Campana, 2014; Carbonara and Follesa, 2019). However, collecting this kind of data has been historically more difficult in cartilaginous fish than bony fish (Campana, 2014; Bellodi et al., 2019). Indeed, the absence in cartilaginous species of the calcified structure mainly used for bony fish ageing, such as otoliths, in addition to the general low calcification level of the cartilaginous structures that often require an artificial increase in growth bands visibility through staining techniques, usually makes cartilaginous fish ageing a rather complex process (Goldman, 2005; Goldman et al., 2012).

Another problem that usually affects cartilaginous fish ageing studies is the inability to obtain sufficient samples, especially for rare or threatened species (Smart et al., 2013). In addition, the acquisition of these species samples could also be affected by legal legislation or conservation issues. For many species, reaching the minimum sample recommended for ageing (200 ca. according to Thorson and Simpfendorfer, 2009) appears unrealistic as they can be difficult to catch due to low abundances (either naturally or driven by human activities) and gear selectivity (Thorson and Simpfendorfer, 2009; Smart et al., 2013).

Moreover, most of the previous ageing studies involved the counting of growth zones without verifying the annual pattern of band deposition (Panfili et al., 2002; Campana, 2014; Carbonara and Follesa, 2019), nonetheless, it must be considered that on some occasions the authors may actually chose not to perform such studies if this kind of analysis has already been conducted on the same species. The analysis of a species growth carried out without verifying the actual band deposition pattern can be affected by the presence of false rings and/or multiple bands on the hard structure, particularly in older specimens (e.g. Campana, 2014; Bellodi et al., 2019; Carbonara et al., 2020), reducing the precision and the accuracy of the age data, and thus affecting eventual stock evaluations (STECF, 2017). In the last decade, the number of ageing studies that include some attempts of verification and validation has increased (Goldman et al., 2012). Many studies are based on semi-direct validation methods, such as the marginal increment analysis, which appears as the methodology most commonly applied for cartilaginous fish (Cailliet and Goldman, 2004; Carbonara et al., 2020), while limited studies have implemented direct validation methods, especially in the Mediterranean (Carbonara et al., 2020).

The thornback ray (*Raja clavata* Linnaeus, 1758) is widely distributed in the North-East Atlantic and the Mediterranean (Ellis et al., 2016), in a large bathymetric range being found at a depth of between 28 and 625 m (Marongiu et al., 2017; Follesa et al., 2019). It is one of the most common elasmobranch species landed in the Mediterranean Sea (Ligas et al., 2013; FAO, 2020). In the basin, the FAO estimated a mean value of 435ca landing tonnes per year in the period between 2010 and 2019, showing also a substantial increase in the last 5 years of the investigated period with a peak in 2019 (924 t) (source FAO Global Fish Trade Statistics; http://www.fao.org/fishery/statistics/en). Nonetheless, this situation led the International Union for Conservation of Nature to list the thornback ray as Nearly Threatened (Ellis et al., 2016), urging the need for updated information on which reliable conservation and management measures can be proposed. Until now, studies concerning the ageing and growth parameters for this species are still insufficient. Holden (1972), Holden and Vince (1973), Brander and Palmer (1985), Gallagher et al. (2005) in the Irish Sea and Whittamore and McCarthy (2005) along Welsh coasts provided growth parameters analysing thornback ray whole vertebral *centra*, while Serra-Pereira et al. (2008) used caudal thorns to describe the species growth along Portuguese coasts. All the studies carried out in the Mediterranean basin, employed vertebral *centra* thin sections and have been conducted in the Strait of Sicily (Cannizzaro et al., 1995), Gulf of Gabes (Kadri et al., 2014) and South Adriatic (Carbonara et al., 2020). In the Mediterranean Sea, the only study that performed a semi-direct validation method was published by Kadri et al. (2014). Kadri and co-authors

(2014) hypothesized the formation of the opaque growth band between September and October, whereas, in Atlantic waters, its formation was reported during the autumn-winter period in the Irish Sea and Bristol Channel (Holden and Vince, 1973) and spring-summer along Portuguese coasts (Serra-Pereira et al., 2008).

While along Atlantic coasts some studies validated the annual deposition of growth bands for this species with chemical tagging (Holden and Vince, 1973; Ryland and Ajayi, 1984, on captive specimens), no age validation studies have been conducted in the Mediterranean Sea since Carbonara et al. (2020). Indeed, the latter study validated, for the first time for this species in the basin, the ageing criteria and ageing scheme through semi-direct (marginal analysis) and, especially, through direct method (chemical tagging).

According to these findings, the present work aims to evaluate the species growth in a wider area of the central-western Mediterranean Sea, using a validated protocol.

Data from four different FAO - GFCM Geographic Sub-Areas (GSAs) were collected to investigate possible differences among them. The obtained result will establish the first evaluation of a cartilaginous fish species growth in such a wide area of the Mediterranean Sea, potentially representing a step towards a finer understanding of the mechanics that regulate the species growth. Moreover, the present study, besides the commonly employed von Bertalanffy function (von Bertalanffy, 1938), applied several alternative models to age-at-length data in consideration also that recent papers stated that often alternative growth models could offer a better fit to cartilaginous fish age-length data (e.g., Neer and Thompson, 2005; Fisher et al., 2013), in particular, for batoids (e.g. Mejía-Falla et al., 2014; Bellodi et al., 2017, 2021).

2. Materials and methods

Samples were collected in four Central-Western Mediterranean Sea FAO-GFCM Geographical subareas (Fig. 1) between 2016 and 2019 from the international experimental trawl survey project MEDITS (MEDiterranean International Trawl Survey; Spedicato et al., 2019), and during biological sampling of commercial fishery (Data Collection Framework program, Reg. (EU) 2017/1004). Each specimen sex was recorded and its total length (TL) was measured to the nearest 0.1 cm.

A part of the vertebral column was extracted from the abdominal cavity of each thornback ray (6–10 *centra*). Neural and haemal arches were detached from each *centrum* using a scalpel. Following Goldman (2005), *centra* were then soaked in a 5% sodium hypochlorite solution for 5–10 min (depending on their size) in order to remove excess of soft tissues, immediately after, all *centra* were soaked in distilled water for about 30 min. Cleaned *centra* were stored frozen.

Vertebral *centrum* sagittal sections were used in the age estimation process. The whole *centra* were embedded in a bi-component epoxy resin (PROCHIMA E-30). After this process, thin sections (500 µm) were obtained using a low speed diamond wheel saw (REMET SECOTRON 200) with a micrometric knob. In order to obtain optimal band visibility, sections were then sanded with a polisher (ATM Saphir 320) equipped with 1000 grit abrasive disks. No staining methods were applied since the contrast between growth bands appeared sufficiently high. The vertebral *centra* sagittal section of each specimen was photographed through a stereomicroscope (LEICA S9i) connected to a Leica Application Suite. In order to discriminate the bands on the vertebrae and facilitate their interpretation, each digital image was converted to greyscale and the contrast and sharpness increased in accordance with Campana (2014), using the software Adobe Photoshop CS6.

Two experienced readers were used in the ageing procedure, without knowing the specimen sex or size. In the case of no agreement between the readers, the *centrum* was read a third time by the two readers together. In this case, if still no accordance was reached, the *centrum* was discarded as not easily interpretable. Bands were counted starting from the first easily recognizable band pair after the first clear mark corresponding to an angle change in the c*orpus calcareum* defined as the birth
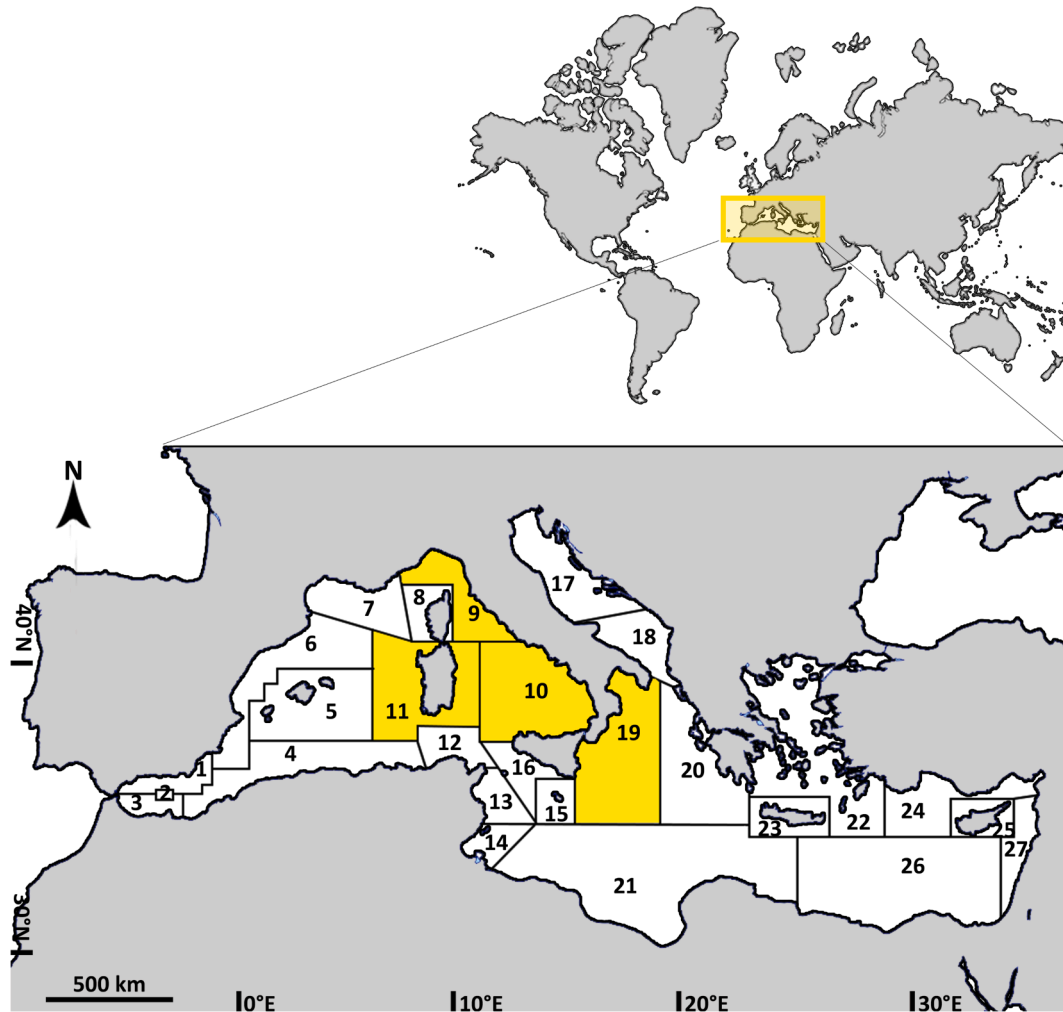
Fig. 1. Map of the Mediterranean Sea FAO_GFCM geographical sub-areas (GSAs). The GSAs involved in the present study are highlighted in yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mark (BM) (Sulikowski et al., 2003) (Fig. 2).

The age was assigned to each specimen applying the ageing scheme for cartilaginous fish provided by Carbonara and Follesa (2019). The 1st of January was considered as the birth date, following the common protocol for species with multiple spawning periods, like the thornback ray (Carbonara and Follesa, 2019 and references therein). According to Carbonara and Follesa (2019), the following criteria were applied to estimate the fish age:

- only transparent rings (perceptible as bands in the *centrum* sections) were counted starting from the very first one laid down after the birthmark;
- deposition of one opaque and one transparent ring (or band in the *centrum* section) per year;
- transparent rings should be observable in all *corpus calcareum* arms to be counted;
- the distance between winter rings should decrease with increasing age.

The coefficient of variation CV% (Chang, 1982), the index of average percent error IAPE (Beamish and Fournier, 1981) and the percentage of agreement A% were calculated in order to evaluate the overall accuracy of the readings. A Multi-Model Inference analysis (MMI) was assembled and four different growth models were applied to the age-at-length data by sex and area (GSA9, 10, 11, 19). The von Bertalanffy growth model both with t_0 formulation (3VBGF; $TL = L_\infty(1 - e^{-k(t-t_0)})$) (von

Bertalanffy, 1938) and with L_0 formulation (2VBGF; $TL = L_\infty - (L_\infty - \beta) e^{[-(kt)]}$) (Fabens, 1965) was applied to the data. Additionally, two more models were applied offering a sigmoid curve, to describe the hypothetical two-phase growth scenario: the Gompertz ($TL = L_\infty e^{e^{-k(t-I)}}$) (Winsor, 1932) and the Logistic ($TL = L_\infty/1 + e^{-k(t-I)}$) (Richards, 1959) functions. In these equations L_∞ denotes the species theoretical maximum length; k stands for the growth rate coefficient; t is the observed age, t_0 represents the hypothetical age of a specimen with TL = 0; β is the result of the following equation $(L_\infty - L_0)L_\infty^{-1}$; L_0 represents the theoretical size at birth or at age 0 (the L_0 value for the present study was set at 11.5 cm as the size of the smallest individual observed for both sexes), and I is the age value corresponding to the curve inflection point.

The fitting level of each growth model to the observed age-at-length data was evaluated through the Akaike's Information Criterion (AIC; Akaike, 1974; Haddor, 2001). Moreover, considering that AIC precision could be negatively affected by the sample size, the corrected Akaike's Information Criterion (AICc) was employed as second-order information criteria to assess model performance (Burnham and Anderson, 2002). Finally, the difference between the AICc value obtained from each model and the lowest AICc value detected was employed to calculate the ΔAIC, and AICc Weight which expresses the likelihood of the model to be the most precise (Burnham and Anderson, 2002; Chin et al., 2013). The best-performing model must exhibit the lowest AICc value in addition to the highest AICc weight. All statistical analysis were carried out in R (R Development Core Team 2012; ver. 4.0.5) using the FSA (R
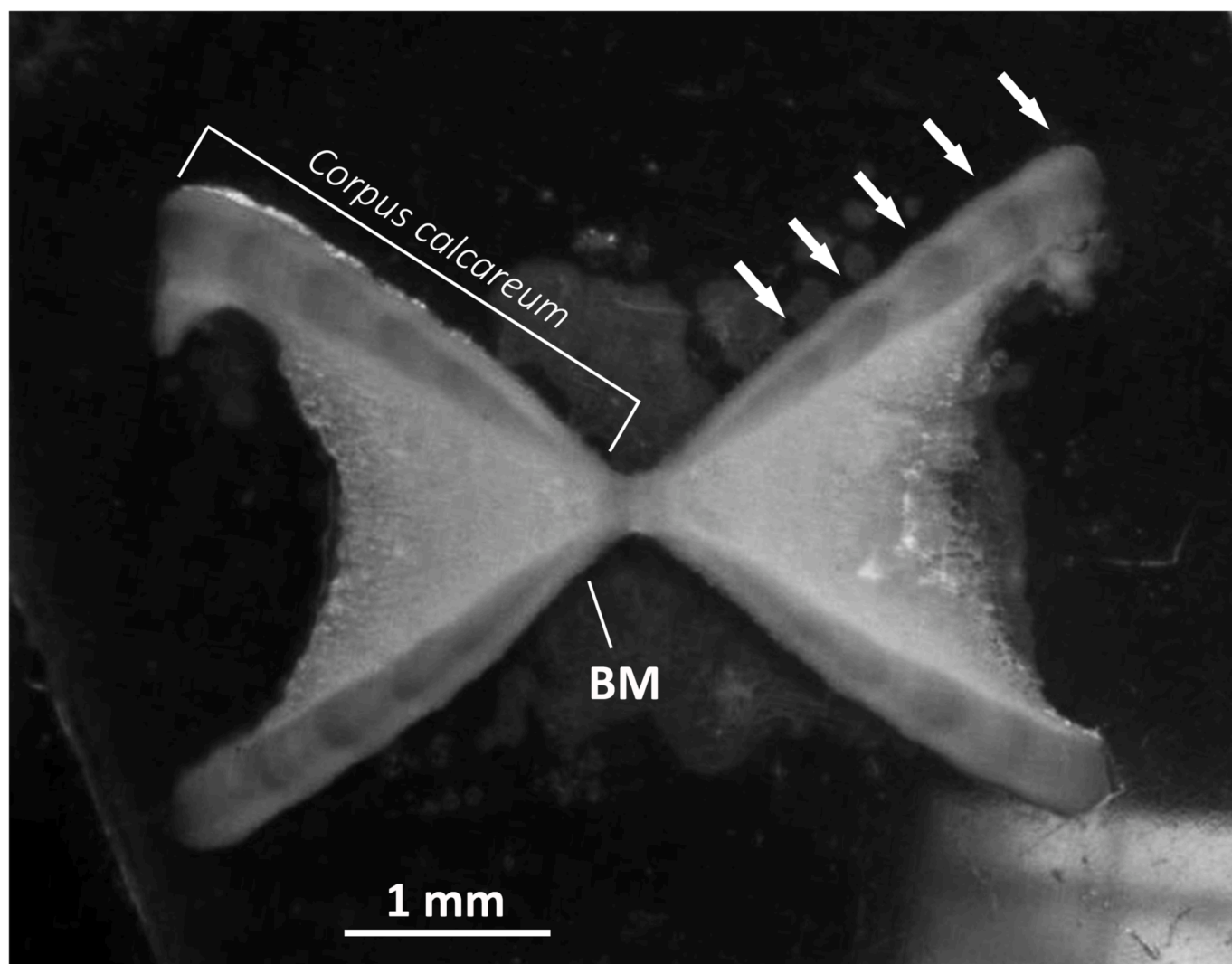
Fig. 2. Vertebral section of *R. clavata* (female, TL = 57.8 cm). White arrows indicate translucent bands. BM = birthmark.

package version 0.8.25, Ogle et al., 2019) and AICcmodavg (R package version 2.3–1, Mazerolle, 2020) packages. Finally a Chen test (Chen et al., 1992) was used to investigate any differences in growth between sexes and areas.

3. Results

A total of 720 thornback ray vertebral *centra* were examined (358 females and 362 males). TLs ranged between 11.5 and 86.4 cm. All the information on the sample composition in each investigated GSA are included in Table 1.

The length-frequency distribution of thornback ray samples showed a good representation of the majority of length classes in all GSAs

Table 1
Sample composition of *Raja clavata*, from the western central Mediterranean Sea by FAO_GFCM geographical sub-area (GSA).

Area		n	n females	n males	TL range (cm)
GSA9	Ligurian Sea and Northern Tyrrhenian Sea	262	126	136	21.5–80.0
GSA10	Southern and Central Tyrrhenian Sea	118	60	58	11.5–86.4
GSA11	Sardinian Seas	235	121	114	13.1–82.4
GSA19	Western Ionian Sea	105	51	54	11.5–82.6

(Fig. 3), with only one exception in GSA10, due to the lack of specimens in the size range of 20–35 cm in TL.

The ageing of *R. clavata* through the analysis of vertebral thin sections emerged as a relatively easy and highly precise process. The ageing readers could easily interpret the growth patterns on the sections and needed to re-read less than 5% of the analysed structure in order to find accordance, while only 11 *centra* were discarded due to the inability of the readers to find an agreement. As a result, the age estimation process achieved a high level of reproducibility and precision (IAPE =4.26; CV% =5.9; PA% = 88.3).

In GSA9 no newborn specimen (age 0) was found, since the minimum age estimated in this area was 1. The older individuals caught in GSA 9 and 10 were aged 11 for both sexes, while the oldest specimens were found in GSA11 (where the oldest female and male were aged 16 and 13 respectively) and GSA19 (where the oldest female and male were aged 13 vs 10 respectively).

The MMI results are shown in Table 2. In general, the lowest AICc values and the highest AICc weight values allowed the 3VBGF to be identified as the model which achieved the highest likelihood level to the observed age-length data, resulting as the most accurate in describing the growth of the species. This outcome was found for males, females and combined sexes in 3 of the 4 investigated GSAs, with the exception of GSA9 (Table 2). In this area, the Logistic model appeared to be the most accurate in describing the species growth. In general, the
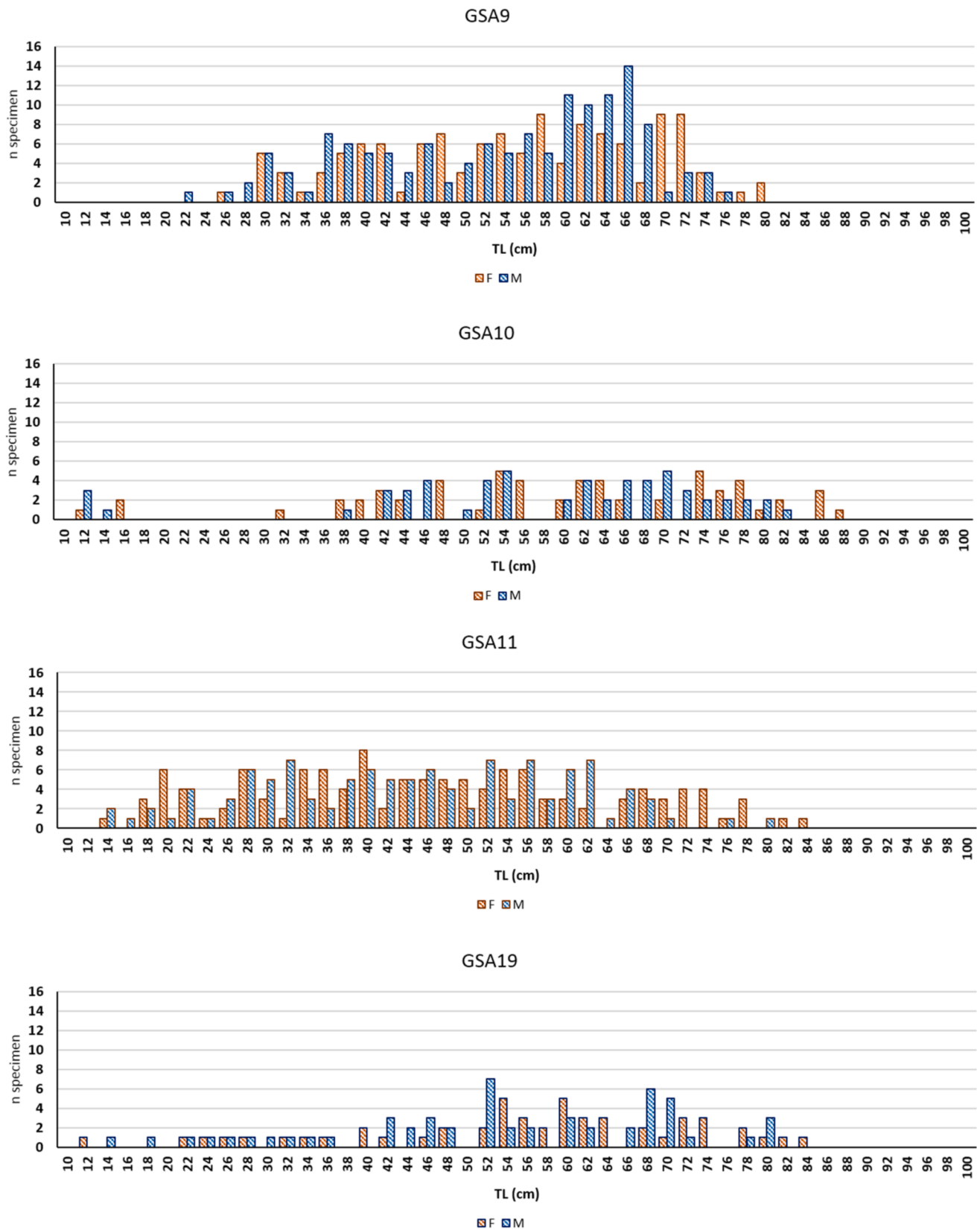
Fig. 3. Length frequency distributions of *R. clavata* in the investigated FAO GFCM Geographic Sub-Areas (GSAs).

3VBGF logarithmic curve performed better than sigmoidal curves in terms of AICc and AICc weights followed by the 2VBGF model as the second best result. The only exception to the last statement was found in GSA11 in which the Gompertz was the second best model, particularly

for females (Table 2).

The Chen test revealed statistically significant differences (Fcalc>Fcrit) in growth between sexes in all the investigated GSAs with the exception of GSA9 (Fcalc<Fcrit). According to the majority of the

Table 2

R. clavata growth parameters (mean ± S.E.) calculated from four models for combined sexes and for females and males separately in each GSA.

	Sex	Growth function	L_∞	k	t_0	I.P.	AIC	AICc	ΔAICc	AICc Weight
	Combined	3VBGF	77.08 ± 2.16	0.27 ± 0.03	−0.47 ± 0.17	–	1629.45	1629.60	6.47	0.024
		2VBGF	78.54 ± 1.74	0.25 ± 0.01	–	–	1628.42	1628.51	5.38	0.041
		Gompertz	73.05 ± 1.46	0.41 ± 0.03	–	1.09 ± 0.08	1624.21	1624.36	1.23	0.328
		Logistic	**70.92 ± 1.16**	**0.55 ± 0.04**	**–**	**1.90 ± 0.08**	**1622.97**	**1623.13**	**0**	**0.607**
	Females	3VBGF,	78.30 ± 3.32	0.26 ± 0.04	−0.41 ± 0.28	–	808.40	808.73	3.27	0.083
		2VBGF	80.32 ± 2.80	0.23 ± 0.02	–	–	807.17	807.17	1.71	0.182
		Gompertz	74.21 ± 2.23	0.40 ± 0.05	–	1.18 ± 0.13	805.80	806.13	0.67	0.306
		Logistic	**72.16 ± 1.76**	**0.54 ± 0.053**	**–**	**2.01 ± 0.12**	**805.13**	**805.46**	**0**	**0.428**
	Males	3VBGF	75.22 ± 2.69	0.28 ± 0.03	−0.46 ± 0.21	–	823.24	823.54	4.21	0.062
		2VBGF	76.63 ± 2.15	0.25 ± 0.02	–	–	821.82	822.00	2.67	0.133
		Gompertz	71.42 ± 1.84	0.42 ± 0.04	–	1.00 ± 0.10	820.07	820.37	1.04	0.300
GSA9		**Logistic**	**69.32 ± 1.44**	**0.57 ± 0.05**	**–**	**1.79 ± 0.09**	**819.02**	**819.33**	**0**	**0.505**
	Combined	**3VBGF**	**92.96 ± 2.91**	**0.21 ± 0.02**	**−0.73 ± 0.11**	**–**	**674.34**	**674.69**	**0**	**0.520**
		2VBGF	91.33 ± 2.36	0.23 ± 0.01	–	–	674.73	674.93	0.24	0.461
		Gompertz	83.96 ± 1.73	0.39 ± 0.02	–	1.27 ± 0.08	681.01	681.36	6.67	0.019
		Logistic	80.73 ± 1.43	0.54 ± 0.03	–	2.14 ± 0.09	693.83	694.19	19.5	0.000
GSA10	Females	**3VBGF**	**95.75 ± 4.39**	**0.20 ± 0.03**	**−0.82 ± 0.18**	**–**	**347.55**	**348.27**	**0**	**0.518**
		2VBGF	93.06 ± 3.26	0.22 ± 0.02	–	–	348.59	349.03	0.76	0.354
		Gompertz	86.57 ± 2.58	0.36 ± 0.03	–	1.30 ± 0.12	350.51	351.23	2.96	0.118
		Logistic	83.25 ± 2.08	0.51 ± 0.04	–	2.24 ± 0.13	355.53	356.26	7.99	0.010
	Males	**3VBGF**	**89.16 ± 3.90**	**0.23 ± 0.03**	**−0.63 ± 0.14**	**–**	**332.01**	**332.76**	**0**	**0.608**
		2VBGF	88.53 ± 3.41	0.24 ± 0.02	–	–	333.21	334.66	1.9	0.235
		Gompertz	80.38 ± 2.27	0.43 ± 0.03	–	1.23 ± 0.11	334.78	335.53	2.77	0.152
		Logistic	77.26 ± 1.88	0.61 ± 0.05	–	2.03 ± 0.12	341.57	342.33	9.57	0.005
	Combined	**3VBGF**	**87.61 ± 2.97**	**0.14 ± 0.01**	**−1.79 ± 0.14**	**–**	**1304.62**	**1304.79**	**0**	**0.994**
		2VBGF	75.11 ± 1.53	0.21 ± 0.01	–	–	1410.04	1410.14	37.58	0.000
		Gompertz	78.25 ± 1.75	0.23 ± 0.01	–	1.24 ± 0.11	1332.25	1332.42	10.17	0.006
		Logistic	74.53 ± 1.38	0.33 ± 0.02	–	2.61 ± 0.14	1359.06	1359.24	19.86	0.000
	Females	**3VBGF**	**89.31 ± 3.29**	**0.13 ± 0.01**	**−1.73 ± 0.16**	**–**	**656.53**	**656.87**	**0**	**0.994**
GSA11		2VBGF	78.78 ± 2.04	0.20 ± 0.01	–	–	721.38	721.59	37.58	0.000
		Gompertz	80.37 ± 1.96	0.23 ± 0.01	–	1.91 ± 0.12	671.64	671.98	10.17	0.006
		Logistic	76.91 ± 1.58	0.33 ± 0.02	–	2.74 ± 0.16	687.50	687.85	19.86	0.000
	Males	**3VBGF**	**80.01 ± 4.26**	**0.15 ± 0.02**	**−1.66 ± 0.28**	**–**	**640.22**	**640.58**	**0**	**0.994**
		2VBGF	68.99 ± 1.95	0.24 ± 0.01	–	–	677.94	6678.16	37.58	0.000
		Gompertz	71.79 ± 2.47	0.26 ± 0.02	–	0.93 ± 0.14	650.37	650.75	10.17	0.006
		Logistic	68.35 ± 1.88	0.38 ± 0.03	–	2.13 ± 0.18	660.08	660.44	19.86	0.000
	Combined	**3VBGF**	**87.05 ± 2.36**	**0.19 ± 0.01**	**−0.88 ± 0.12**	**–**	**551.40**	**551.80**	**0**	**0.536**
		2VBGF	85.12 ± 1.77	0.21 ± 0.01	–	–	551.87	552.10	0.3	0.462
		Gompertz	79.71 ± 1.50	0.33 ± 0.08	–	1.32 ± 0.07	562.69	563.09	11.29	0.002
		Logistic	76.77 ± 1.25	0.46 ± 0.02	–	2.34 ± 0.10	578.88	579.28	27.48	0.000
	Females	**3VBGF**	**84.52 ± 2.50**	**0.21 ± 0.02**	**−0.68 ± 0.16**	**–**	**258.09**	**258.62**	**0**	**0.998**
GSA19		2VBGF	84.65 ± 2.09	0.21 ± 0.01	–	–	260.09	290.67	32.05	0.000
		Gompertz	79.14 ± 1.83	0.34.0.02	–	1.33 ± 0.11	270.39	271.28	12.66	0.002
		Logistic	76.88 ± 1.65	0.47 ± 0.04	–	2.32 ± 0.13	280.94	281.82	23.2	0.000
	Males	**3VBGF**	**89.65 ± 4.51**	**0.17 ± 0.02**	**−1.03 ± 0.19**	**–**	**290.07**	**290.89**	**0**	**0.566**
		2VBGF	85.44 ± 2.99	0.20 ± 0.01	–	–	291.87	292.32	1.43	0.277
		Gompertz	79.87 ± 2.44	0.32 ± 0.03	–	1.31 ± 0.12	292.76	293.57	2.68	0.148
		Logistic	76.19 ± 1.89	0.46 ± 0.03	–	2.31 ± 0.14	298.56	299.38	8.49	0.008

L_∞ = maximum asymptotic length; k = growth coefficient; t_0 = theoretical age at which size equals zero; I.P. = Inflection Point; AICc = corrected Akaike's Information Criterion; ΔAICc = difference between the model value and the lowest obtained AICc value; AICc Weight = likelihood of the model. Values of the best fitting model are highlighted in bold.

investigated models, males are characterized by higher growth rate k than females, which generally presented higher L_∞, with the exception of GSA19 (Table 2). The growth rate k obtained from 3VBGF ranged between 0.27 and 0.19 (considering combined sexes) in GSAs 9, 10 and 19, while in GSA11 this value was 0.14. In contrast, GSA9 appeared as the area in which the species achieved faster growth rate and therefore lower asymptotic length (Table 2). The growth curves obtained from each single GSA were found to be statistically different from each other (Chen test; Fcalc>Fcrit). Moreover, the pairwise test conducted separately for females and males from each different GSA always revealed statistically significant differences among all the GSAs with the exception of females from GSA9 and GSA19 which appeared to share similar growth curves (Chen test; Fcalc<Fcrit). The comparison of the species growth among GSAs is reported in in supplementary material S1. In Fig. 4, the observed age-length data and the computed 3VBGF curves are present, while detailed information on age-at-length data for each GSAs are provided as supplementary material in Fig. S2.
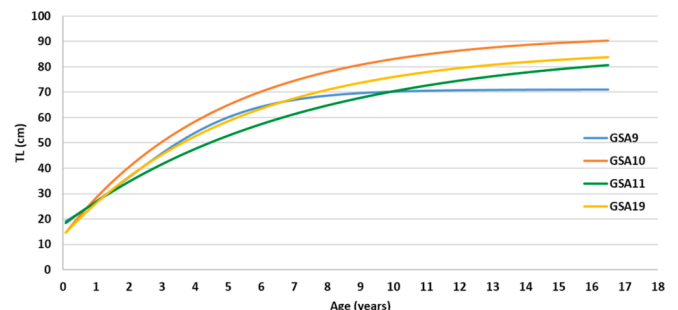
Fig. 4. *R. clavata* growth model for combined sexes based on the 3VBGF, in FAO GFCM Geographic Sub-Area (GSA) 10, 11 and 19, and based on the Logistic model for GSA 9.

4. Discussions

The ageing analysis of thornback ray based on the readings of vertebral *centra* thin sections appeared to be a highly precise and accurate analysis, confirming what has already been reported in literature (e.g. Kadri et al., 2014; Carbonara et al., 2020). The band visibility is generally high and therefore the growth pattern interpretation is relatively easy for the readers, with very few structures being excluded. The high level of training and expertise of the readers may have also played a role. Indeed, the readers' experience has been recognized as one of the key factors driving the precision of ageing studies (Carbonara et al., 2018, 2019). The ageing precision evaluation indexes (IAPE and CV%) showed optimal results in terms of reproducibility and accuracy of the reading process, being well below the threshold value (CV% < 10) proposed as acceptable for cartilaginous fish by Campana (2014). These results were in line with what has been reported for the thornback ray in the Southern Adriatic Sea (IAPE = 3.42–3.87, CV% = 4.5; Carbonara et al., 2020), and Tunisian Waters (IAPE = 3.42, CV% = 6.69; Kadri et al., 2014). Moreover, the confirmation of the annual band deposition pattern, described for this species in the Mediterranean Sea (Kadri et al., 2014; Carbonara et al., 2020) and Atlantic waters (Holden and Vince, 1973; Serra-Pereira et al., 2008) and, especially, the direct age validation through chemical tagging conducted in an adjacent area (Carbonara et al., 2020) contributed to strengthen further the reliability of the ageing protocol adopted in the present study.

In light of these results, the analysis of vertebral *centra* thin sections should be considered as one of the most reliable methods to investigate *R. clavata* growth. Some other structures and methods have been proposed for ageing this species, such as caudal thorns used in the evaluation of the species growth along the Portuguese coasts (Serra-Pereira et al., 2008). The extraction of these structures does not necessarily require the sacrifice of the animal, moreover, they can also be sampled at fish auctions without buying the entire specimen. However, considering that such structures can be lost or damaged during the specimens' life they may not precisely represent the entire life spawn of the animal, adding a possible bias to age analysis (Meunier and Panfili, 2002). Whittamore and McCarthy (2005) employed whole stained vertebral *centra* along Welsh coasts, while Carbonara et al. (2020) stained the whole *centra* in cobalt nitrate and ammonium sulphide (Hoenig and Brown, 1988; Goldman, 2005) proving this method to be highly reliable even if less precise than thin sections. Also Başusta et al. (2017) reported interesting results in increasing thornback ray's growth bands visibility with staining methods (e.g. Silver Nitrate; Crystal Violet).

Although using whole *centra* does not require embedding and cutting procedures, thus saving operational time and costs of equipment and consumables, we consider the use of thin sections as the most precise and accurate method for ageing thornback ray, as reported by Carbonara et al. (2020). Moreover, the thin section preparation method without any staining provided an adequate level of precision (ICES, 2011).

In recent years, the use of multiple growth models in the analysis of cartilaginous fish growth has been strongly recommended (Katsanevakis and Maravelias, 2008; Smart et al., 2016). The common 3VBGF was not always indicated as the most reliable in modelling the growth of cartilaginous fish and especially batoids, which usually abruptly slow their growth in size while continuing to increase in weight (Fisher et al., 2013). Liu et al. (2015) identified three groups of cartilaginous fish based on their growth pattern and biological parameters. The first group comprised large sized sharks with a long life span and slow growth rate. On the other hand, the second group included small sized species that grow rapidly with a short life span. Finally, the third group encompasses late-maturing sharks characterized by a moderate life span. According to this scheme, skates and rays usually belong to the second group and generally the sigmoidal models should provide the best fit to their growth (Liu et al., 2015, 2021). Although the last assumption has proven to be true for several batoids (e.g. Fisher et al., 2013; Mejía-Falla et al., 2014; Bellodi et al., 2017, 2021; Porcu et al., 2020), *R. clavata* seemed to

represent a slight exception to those reported by Liu et al. (2021). Indeed, in general, the MMI results obtained in the present study indicate the 3VBGF as the most appropriate for modelling thornback ray growth in terms of fitting to the age-length data. This is in line with the results obtained by Carbonara et al. (2020) and Kadri et al. (2014), even if in the latter study the 3VBGF was only tested against the Gompertz model. However, among all the investigated areas, only in GSA9 the AICc and AICc weights have indicated the Logistic as the best fitting model. This result could be related to the fact that, despite this GSA attained the highest sample number, its length structure appeared slightly skewed towards larger sizes. In this regard, the Logistic model fitting result could have been boosted by this peculiar sample composition. This outcome could have also been driven by the absence in the GSA9 of new born individuals (age 0) below 21.5 cm in TL. However, considering that GSA 10 and 19, in which the 3VBGF was the best fitting model, also presented some gaps in the sampling of the smaller size classes, the last hypothesis seemed to be rejected. The thornback ray appears as a medium-sized batoid characterized by a moderately slow growth rate, which gradually slows down during the animal life span without any sign of abrupt changes that typically occurs in two-phase growing species that usually present faster growth rates such as, for example, the speckled ray (k = 0.26; Porcu et al., 2020) that also inhabits the same areas, or other non-rajid batoids as the Atlantic cownose ray a (k = 0.19–0.27), (Fisher et al., 2013).

All the available studies carried out on the thornback ray reported females attaining larger sizes but growing more slowly than males, both in the Atlantic (Gallagher et al., 2005; Whittamore and McCarthy, 2005; Serra-Pereira et al., 2008) and Mediterranean waters (Cannizzaro et al., 1995; Kadri et al., 2014). This pattern, commonly reported in cartilaginous fish (e.g. Sulikowski et al., 2007; Mulas et al., 2015; Porcu et al., 2015), is confirmed also by the present study, where males were found able to grow significantly faster than females, but attaining smaller maximum sizes, with the only exception of GSA19 where males achieved significantly larger L_∞ and lower k values than females However, this difference might be ascribed to the relatively low number of specimens caught in that area. In GSA 9, no statistically significant differences were found between sexes. The same situation was reported by Carbonara et al. (2020) in another FAO_GFCM geographic sub-area (GSA18). In this regard, considering that the present study and the one by Carbonara et al. (2020) shared the very same methods, the presence/absence of significant differences in growth between sexes could be related to the peculiar eco-environmental features that characterize the investigated area. However, this aspect needs to be investigated further in order to be clarified.

Additionally, in GSA9 the species showed the fastest growth rate, however, it must be considered that this value comes from the Logistic model which tends to estimate higher growth rates than the logarithmic models. For this reason, further analysis should be performed in order to clarify this aspect in GSA9, also searching for juvenile specimens that might concentrate in specific nursery areas (Cau et al., 2017). The slowest growth rate observed in GSA11 is similar to those reported for this species in other contiguous areas, such as Tunisia (Kadri et al., 2014) and Sicily (Cannizzaro et al., 1995), suggesting the possibility of a different growth pattern of the species in the south-west Mediterranean regions. Indeed, it is well reported in literature that different environmental conditions (Girard and Du Buit, 1999; Carbonara et al., 2018) or fishing pressure (Aranha et al., 2009) may lead to differences in growth rates in adjacent areas. In GSA10 and GSA19, the species showed growth parameters similar to those reported by Carbonara et al. (2020) in the southern Adriatic Sea (GSA18). In general, the thornback ray growth parameters available in literature seem quite heterogeneous and do not reveal any clear pattern with respect to the investigated area latitude or longitude. For example, the species estimated growth rate ranged from 0.08/0.09 in North Wales (Lesser, 1967) and the Sicilian Channel (Cannizzaro et al., 1995) to 0.18 also in North Wales (Whittamore and McCarthy, 2005) or 0.21–0.27 in the present study in GSA10 and GSA9

(even if the latter comes from the Logistic equation). Moreover, much higher k values are reported in literature such as 0.46 from Algerian waters (Adda-Hanifi et al., 2017), even though it must be considered that this value came from length–frequency distribution analysis LFDA. The age estimation method, whether by direct growth band counting or indirect analysis could certainly be a cause of discrepancies between obtained parameter among areas (Carbonara et al., 2019). In the same way the used structure or the structure preparation could also lead to this outcome (Carbonara et al., 2019). However, considering that the present study applied the same protocol to four adjacent areas, the observed difference in thornback ray's growth areas highlighted the urgent need to investigate this aspect on a wider geographic scale, searching for possible differences at sub-region level. In this regard, future studies that aim to investigate cartilaginous fish growth in different areas should also consider those environmental factors that may cause metabolic, and consequently, somatic growth differences. For example, the nutrients density in a certain area could affect the entire food web up to the top predator level (Wetherbee et al., 2012; Mulas et al., 2019), boosting or weakening their growth rate. Moreover, several other environmental factors, such as the pollution level or any other anthropogenic disturbance (Aranha et al., 2009; Bellodi et al., 2021), may also affect a species growth rate in a certain area. Future studies that succeed in integrating such approaches should be able to provide highly reliable information on the age structure of the populations for an adequate evaluation of the stock status and their effective management.

The outcomes of the present study describe thornback ray as a batoid capable of growing relatively fast during the first years, with a gradual decline in growth with increasing age, perfectly fitting in the batoid's group of the cartilaginous fish growth-type grouping sensu Liu et al. (2015, 2021). However, differently from what has been reported by Liu et al. (2021), which indicated that usually sigmoidal models are the most precise in describing the growth of batoid species, the 3VBGF proved to be the best model to describe the thornback ray growth.Hence such an outcome indicates this species as a slight exception to the cartilaginous fish growth-type grouping proposed. This further stresses the importance of using a MMI approach in future ageing studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.seares.2021.102141.

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