ICES CM 2005/N:17 - Elasmobranch Fisheries Science

# Age and growth of *Raja clavata* Linnaeus, 1758 – evaluation of ageing precision using different types of caudal denticles

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## Abstract

Dermal denticles are useful tools in taxonomic and phylogenetic studies focusing Chondrichthyes. In particular, Rajidae species can be differentiated, among other characteristics, by the number, disposition and morphology of these structures along the dorsal surface. Three types of dermal denticles can be defined: prickles, thorns and bucklers. The first ones are found all over the body, while, the remaining are present on restricted body locations. On a recent work, caudal thorns were successfully used for ageing rays and skates from Falkland Islands, but seemed inappropriate to age Atlantic species. The present paper analyses different types of denticles from the caudal region of *Raja clavata*, using specimens caught in Portuguese waters, with total length ranging from 13 to 89 cm. Denticles from each group were further analyzed for ageing purposes. The distance from the *centrum* origin to the distal edge of each growth band and the band width were measured. Following pre-established criteria on ageing reproducibility, thorns were selected and ages were assigned for each sampled specimen. Ageing precision was compared between thorns and vertebral *centra*. According to statistical measures thorns revealed to be more precise. Von Bertalanffy growth model was adjusted to age-at-length data: $L_\infty$= 130.5 cm, k= 0.10 year$^{-1}$ and $t_0$= -0.14 year. Although females attain larger sizes than males, no statistical differences were observed on growth rates between sexes.

**Keywords**: *Raja clavata*, dermal denticles, ageing precision, age, growth, Portugal.

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## Introduction

The impact of the fishing on cartilaginous fish, in marine ecosystems, is currently a subject of an increasing concern among international authorities (Stevens, 2000). This concern is mainly due to the fact that elasmobranchs in general, and rays and skates in particular, are k-strategists characterized by a slow growth rate, a late age at first maturity, low fecundity and natural mortality. These characteristics make them particularly vulnerable to fishing activity. However, and although sharing this main feature, rays and skates species show a large variation in their life-history parameters and therefore show differences in their plasticity to changes in the environment.

The thornback ray (*Raja clavata* Linnaeus, 1758) is one of the most sensitive species in the North Sea (Walker & Hislop, 1998), still being abundant in the Northeast Atlantic (Walker, 1999), including the Portuguese waters (Bordalo-Machado et al., 2004). This coastal species has a distribution from shallow waters to 700 m depth, on a variety of substrata, occurring from Norway to South Africa and also in Mediterranean, Baltic and Black Sea (Stehmann & Bürkel, 1984).

Age and growth studies are an important component of fisheries resource assessment, mainly because they are the basis for the calculation of growth and mortality rates, length at first maturity and other important biological variables. In the various age and growth studies developed with elasmobranch species, authors had used length frequency models (Brander &

Palmer, 1985), tagging experiments (Steven, 1936; Holden, 1972) and interpretation of growth bands in calcified structures – vertebral *centra* (Coelho & Erzini, 2002; Carlson & Baremore, 2003), spines (Holden & Meadows, 1962; Bordalo-Machado & Figueiredo, 2000) and neural arches (McFarlane *et al*., 2002). In rays and skates the vertebral *centra* are the structures traditionally used for ageing, through the analysis of translucent and opaque bands (Daiber, 1960; Du Buit & Maheux, 1986; Cailliet, 1990), being already validated for *Raja clavata* (Holden & Vince, 1973; Ryland & Ajayi, 1984). Recently, (Gallagher & Nolan, 1999) dermal denticles were introduced as a new ageing method for rays and skates being successfully used for ageing *Bathyraja* spp. from Falkland Islands, but indicated as inappropriate to age Atlantic species, in particular *Raja clavata* (Gallagher *et al*., 2002). Until that study, dermal denticles were only used for taxonomic and phylogenetic studies to differentiate Rajidae species, based on the disposition, number and morphology along the dorsal surface (Stehmann & Bürkel, 1984).

The main objectives of this study were: i) identify the different types of denticles on the caudal region of *Raja clavata*, and select the most adequate for age readings; ii) compare the ageing precision between the traditional technique using vertebra and the new technique using dermal denticles; iii) and estimate the age and growth of *Raja clavata,* in Portuguese waters.

## Material and Methods

Sampling

A total of 200 individuals of *Raja clavata* species (115 females and 85 males), with total length ranging from 13 to 89 cm, were sampled along the Portuguese coast. The samples were obtained monthly, between June 2003 and May 2004, from research surveys and from artisanal fleet landings on the two major ports with rays and skates landings: Matosinhos (North) and Peniche (Center) (Figure 1).

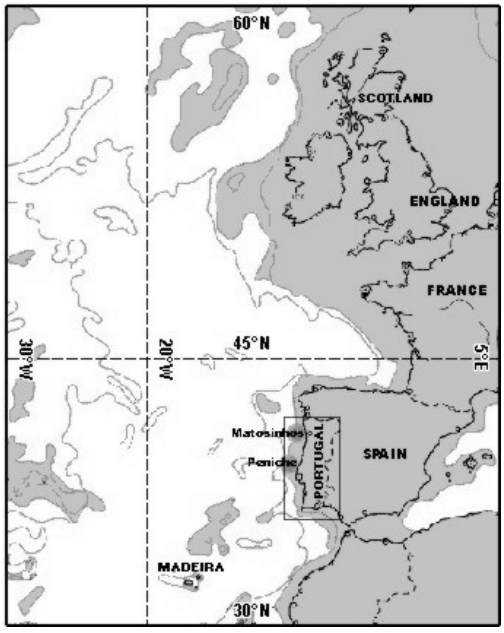


Figure 1. Sampling area: Research surveys along the Portuguese Continental Coast and landing ports of Matosinhos in the North and Peniche in the Center of Portugal.

Each individual was measured to the nearest millimeter total length (TL) and disc width (DW), weighted, sexed and maturity stage assigned according to Stehmann (2002). For age readings, vertebrae were removed from the abdominal region and the tail cut for dermal denticles removal.

Experimental procedure

*Vertebrae*

To remove the adjacent tissues and obtain the vertebral *centra*, the vertebrae were immersed in bleach, during 30 minutes. To eliminate the bleach residuals, they were cleaned with running water and subsequently dried at room temperature, during 24 hours. The remaining tissues in the inner surface were removed with a blade. To enhance the band pattern each *centra* was burned in the oven at 200 ºC, during 10 minutes.

*Dermal denticles*

A piece of the tail with, at least, three dermal denticles, was individualized from each specimen. Each sample was prepared according to the technique adapted from Gallagher & Nolan (1999). The tail pieces were immersed in 5% trypsin solution with phosphate buffer solution with pH 7.5, during 14 hours, in a water bath at 40 ºC, for adjacent tissue removal. To remove the tripsin residues the dermal denticles were cleaned with distilled water, to avoid posterior corrosion. To enhance the band pattern, each individualized dermal denticle was immersed in 5% ethylenediaminetetraacetic acid (EDTA) solution, during 10 minutes.

Ageing procedure

From each specimen, the different types of dermal denticles and vertebral *centra* were observed, using an Olympus S2X9 stereomicroscope, with a magnification between 10x and 48x. The images were digitalized using the image analysis software TNPC 4.1. (Noesis, 2002). Caudal denticles from each different group were further analyzed for ageing purposes. Following pre-established criteria on ageing reproducibility (Campana, 2001), the adequate group was selected and ages were assigned using dermal denticles and vertebrae by 2 independent readers. To analyze their ageing precision three statistical measures were used: Average Percent Error (APE) (Beamish & Fournier, 1981), Coefficient of Variation (V) and Index of Precision (D) (Chang, 1982):

$$APE_j = 100\% \frac{1}{R} \sum_{i=1}^{R} \frac{\left| X_{ij} - X_j \right|}{X_j} \qquad V_j = 100\% \frac{\sqrt{\sum_{i=1}^{R} \left( X_{ij} - X_j{}^2 \right)}}{\frac{R-1}{X_j}} \qquad D = \frac{V}{\sqrt{R}}$$

where $X_{ij}$ is the *i*th age determination of the *j*th fish, $X_j$ the mean age estimate of the *j*th fish, and $R$ is the number of times each fish is aged.

Using TNPC 4.1 (Noesis, 2002), the distance from the *centrum* origin to the distal edge of each growth band and the band width were measured, along an imaginary axis (radial), to identify the variations on growth increment between different structures. The Von Bertalanffy growth curve was fitted to length-at-age data and parameters were estimated for males and females, separately. Hotelling's $T^2$ test (Bernard, 1981) was used to compare the growth parameters between sexes.

## Results

Analyzing the caudal region of *Raja clavata*, based on size and characteristics of the basal plate (BP) and crown, dermal denticles of 3 main types were identified: thorns, bucklers and prickles (Figure 2). Because each type has is own characteristics, not all denticles demonstrated to be adequate for ageing assessment. Thorns (Figure 2.A-B) are located only in restricted body locations, and in the case of the caudal region are found along the median row and on the lateral side; morphologically could be identified 2 different types: a) large basal plate (lBP) and long crown (Figure 2.A) and b) small basal plate (sBP) and long crown (Figure 2.B). Bucklers (Figure 3.C) occur on the lateral surface of the tail; are the larger (8.58±2.14 mm) and have a thick and opaque basal plate that makes the growth bands more difficult to identify. Prickles (Figure 2.D) can be found covering the entire fish dorsal surface, have a very small size (2.32±1.01 mm), various shapes (e.g. cross, star and leaf) and an irregular outline of the basal plate edge that difficult the growth band identification.

Regarding thorns and bucklers, different stages of development were observed: dermal denticles assume modifications in shape, with a sequential growth of the BP in size and thickness. On the present paper the terminology 1st and 2nd stages was used to designate earlier and later stages of development, respectively.

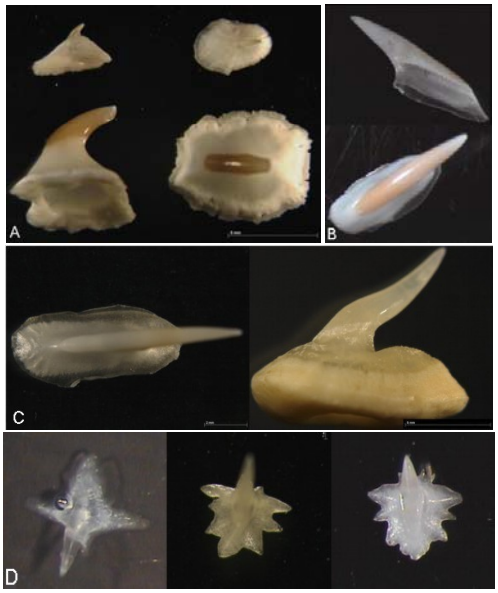


Figure 2. Types of dermal denticles observed in the caudal region of *Raja clavata*: A) Thorns with a large basal plate, on the 1st (above) and 2nd (below) stages of development (10x); B) Thorns with a small basal plate, on the 1st (above) and 2nd (below) stages of development (30x); C) Bucklers on the 1st (left) and 2nd (right) stages of development (15x); D) Prickles: cross, leaf and star (48x).

The occurrence of the different types of dermal denticles along the length classes was analyzed (Figure 3). Until 30 cm TL, thorns with large BP, located on the median region of the tail were the only type of dermal denticles observed. This type of denticles persisted along the entire fish life. At 30-40 cm TL, thorns with small BP start to appear on the median region, representing approximately 12% of the total cover of the tail. Between 40 and 60 cm TL, thorns with large BP on the median region still represented more than 70% of denticles; however, prickles make its appearance and the same happens with; thorns and bucklers on the lateral region of the tail that represented 6% of cover on individuals with 40-50 cm TL and 22% on 50-60 cm TL. At 60-70 cm TL, thorns with large BP represented 56% of the body cover while bucklers on the 1st lateral region represented approximately 30%; bucklers on the

2nd lateral were observed only in this length class. At 70-80 cm TL, thorns with large BP located on the median and 1st lateral regions represented 60% and 13% respectively, while bucklers on the 1st lateral represented 21%. Above 80 cm TL, individuals presented 55% of thorns with large BP and 7% of prickles on the median region, 29% of bucklers and 7% of prickles on the 1st lateral region.

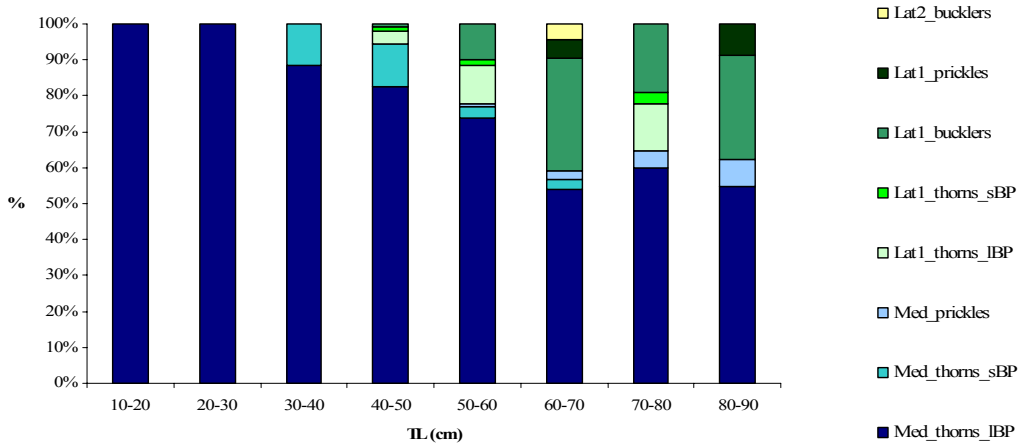


Figure 3. Relative frequency (%) of the different types of dermal denticles identified on the caudal region, by total length classes of 100 mm: Med_thorns_lBP: thorns with large basal plate on the median region, Med_thorns_sBP: thorns with small basal plate on the median region, Med_prickles: prickles on the median; Lat1_thorns_lBP: thorns with large basal plate on the 1st lateral region, Lat1_thorns_sBP: thorns with small basal plate on the 1st lateral region, Lat1_bucklers: bucklers on the 1st lateral region, Lat1_prickles: prickles on the 1st lateral region, Lat2_bucklers: bucklers on the 2nd lateral region. (n=200)

Comparing all the identified dermal denticles types, based on the morphology and on the difficulty to distinguish the translucent and opaque growth bands, it can be stated that the most adequate denticles for ageing *Raja clavata* seems to be the thorns with large BP. In fact, comparing the same type of denticles taken from the same body region of the same individual, there were no great differences between them, presenting the dermal denticles a very similar band deposition as demonstrated in Figure 4.

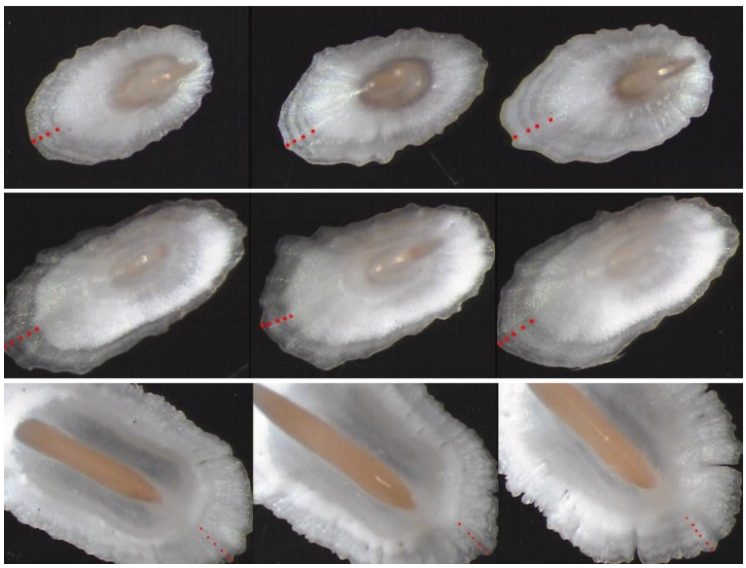

Figure 4. Resemblance between the same dermal denticles type (thorns with large BP) from the same body region of the same individual. From the top to the bottom: a) 450 mm TL, 4-year, b) 513 mm TL, 5-year and c) 654 mm TL, 6-year.

Analyzing caudal thorns and vertebral *centra* from the same individual a great consistency on the age readings was observed (Figure 5). The application of ageing statistical measures on the observations made by different readers(Table I), showed that, for each measure, caudal thorns presented the lower values, indicating that ageing using these structures are more reproducible than with vertebral *centra*.
When ageing the young-of-the-year, 2 formed bands that seemed to correspond to embryonic growth and persisted along the post-embryonic development could be consistently observed (Figure 5).

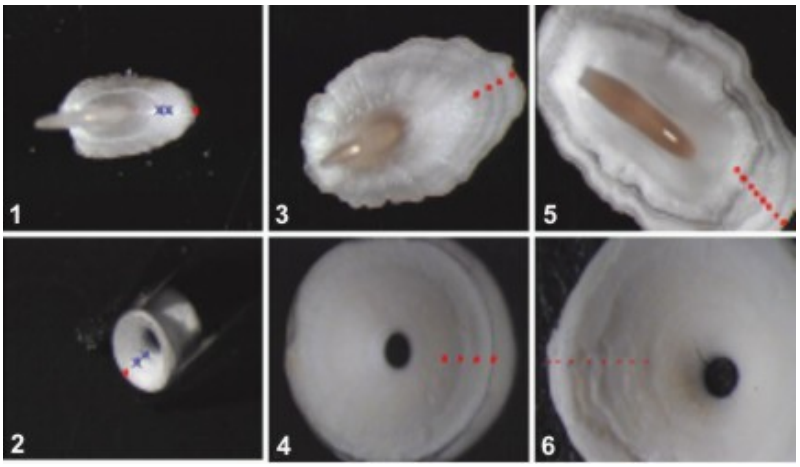


Figure 5. Comparison between caudal thorn and vertebral *centra* from 3 individuals: (1-2) male aged 1-year and 19.9 cm TL; (3-4) male aged 4-year and 44.2 cm TL; (5-6) female aged 8-year and 78.1 cm TL. **x** False bands. • True bands.

Table I. Average Percent Error (APE), Coefficient of Variation (V) and Index of Precision for age readings made by two independent readers, using caudal thorns and vertebral *centra*.

| | %APE | %V | %D | %Agreement Between readers |
|---|---|---|---|---|
| Caudal Thorns | 2,3 | 3,2 | 2,3 | 79 |
| Vertebral *centra* | 8,6 | 12,2 | 8,6 | 57 |

In result of the large morphological variation of the dermal denticles, the measurements made in this study demonstrated great amplitude, even within the same denticles type. In Figure 6 is possible to observe a sequential increment of growth bands along the denticles radial, causing an enlargement of the denticles diameter with age.

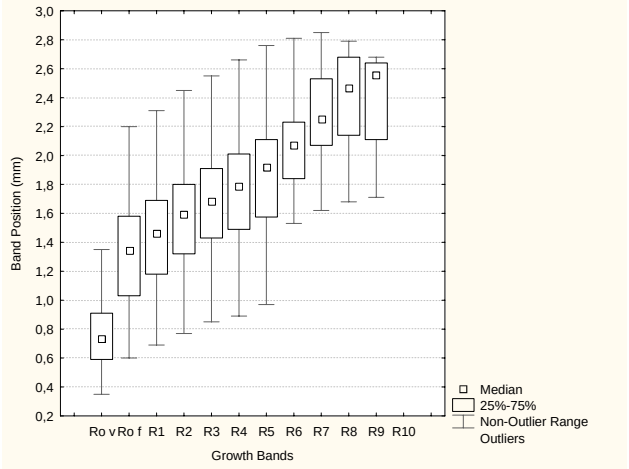


Figure 6. Distribution of growth bands (mm) along the radial, using thorns with large BP.

The Von Bertalanffy growth parameters were estimated for length-at-age data for sex (Table II). Since no statistical differences between sexes were found ($T^2$ = 1.784, $T^2_0$= 9.998; p>0.05), the Von Bertalanffy growth model was estimated for both sexes combined (Figure 7).

Table II. Von Bertalanffy growth parameters of *Raja clavata* from the Portuguese Continental Coast estimated using age-at-length data

| | (cm) | R | $L_\infty$ (cm) | k ($year^{-1}$) | $t_0$ (year) |
|---|---|---|---|---|---|
| All | [13, 89] | 0.99 | 130,5 | 0,10 | - 0.14 |
| Males | [15, 77] | 0.99 | 121,5 | 0.11 | - 0.11 |
| Females | [13, 89] | 0.99 | 130,5 | 0.10 | - 0.13 |

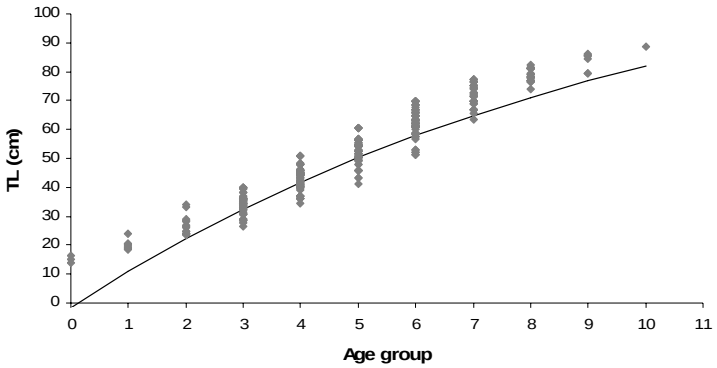


Figure 7. Growth curve of *Raja clavata* on the Portuguese waters.

## Discussion

The different morphological types of dermal denticles (thorns, bucklers and prickles) have already been identified by Capapé & Desoutter (1981), Stehmann & Bürkel (1984), Deynat (1998, 2000), Gravendeel *et al*.( 2002) and considered to be a main characteristic of Rajidae species by.Stehmann & Bürkel (1984). In this paper, we have focused our study on the caudal region of *Raja clavata,* relating the denticles with the growth process of individuals as well as we characterize the differentiation of two morphological thorns types.

In ageing studies, the great or less difficulty on the band observation and identification depends on the analyzed species, on the ageing structure nature (e.g. degree of calcification) (Campana, 2001) and on the applied preparation technique. In this study not all the identified dermal denticles seemed adequate for ageing thornback ray. Only thorns with large basal plate and small crown showed a precise growth band deposition, were easy to identify, and consistent with total length increments along time. The other dermal denticles (thorns with small BP, prickles and bucklers) should not be used for ageing purposes since: a) their morphology difficults growth band characterization; and b) they are not present in specimens smaller than 30 cm TL and therefore do not show growth bands since birth.

To prove the readability of caudal thorns, a comparison of age assignments between thorns and vertebtal *centra* taken from the same specimen was performed. A consistent similarity on ageing assessment was observed. Furthermore, caudal thorns demonstrated a semi-transparent appearance that facilitated the identification of the growth band pattern. Comparing the two experimental procedures, we could say that despite the long duration of the immersion in trypsin of the dermal denticles, the technique was easy to perform. In opposition, the vertebral *centra* technique required a manual "sculpting" to remove the adjacent tissues that remained

after the bleach action, which interacted negatively with age readings. In conformity the statistical ageing precision measures applied to the observations indicated that caudal thorns were more precise than vertebrae, demonstrating their greater reproducibility. According to Campana (2001) elasmobrachs age and growth studies showed a mean coefficient of variation of 10% for vertebral *centra* and 11% for spines. In this study the ageing procedure using caudal thorns was better than the average, presenting mean APE and V values below the precision limits established by Campana (2001): APE lower than 5.5% and V below 7.6%. Gallagher and Nolan (1999), using thorns, obtained V values higher than those of the present paper, with a minimum of 4.2% for *Bathyraja brachyurops*. In the future, caudal thorns could be useful tools for ageing assessment because, in addition to their high precision, they can be removed with minimal damage, without sacrifice of the animal and without alteration of their commercial value.

Table III shows the comparison of the Von Bertalanffy growth parameters (VBGP) for *Raja clavata* estimated in different areas of the NE Atlantic. Variations in life-history traits between geographically separated populations of skates and rays are not unusual, as would be expected from phenotypic plasticity. Analysing this table, one can conclude that VBGP estimated for thornback ray caught in the Portuguese waters are slightly different from those estimated for individuals from the North and Irish Seas: *specimens* attain a larger theoretical maximum size ($L_\infty$) and reach $L_\infty$ at a slower rate (k). The statistical differences between studies were not analyzed because the original data was not accessible. The stated differences could be due to environmental conditions, interpretation of growth bands or to the sampling deficiencies that included individuals smaller than 90 cm, below the maximum size recorded in the other studies. In this paper two false bands situated before age-1 band were considered what have not been reported by other authors. These false bands were already present in specimens with a total length near birth length, which makes us think that these two false bands may correspond to embryonic growth.

Concluding, caudal thorns can be used for *Raja clavata* ageing assessments in Atlantic waters in substitution of vertebral *centra*. However, validation is required through mark/recapture, tetracycline and/or growth monitorization studies to certify the introduction of thorns as an effective tool for skates and rays ageing assessment.

Table III. *Raja clavata* growth parameters estimated by various authors.

| Authors | Area | Sex | n | $L_\infty$ (cm) | k (year$^{-1}$) | $t_0$ (year) |
|---|---|---|---|---|---|---|
| **Current Paper** | **Portugal** | **Both** | **200** | **130.5** | **0.10** | **- 0.14** |
| Walker, 1999 | North Sea | F | 51 | 118 | 0.14 | -0.88 |
| | | M | 41 | 98 | 0.17 | -0.43 |
| Fahy, 1989 | Irish Sea | F | 1504 | 107.8 to 120 | 0.15 to 0.26 | -1.01 to 0.05 |
| | | M | 783 | 96.8 to 104.3 | 0.19 to 0.24 | -1.36 to 0.32 |
| Brander & Palmer, 1985 | Irish Sea | Both | 1125 | 105 | 0.22 | 0.45 |
| Ryland & Ajayi, 1984 | Bristol Channel | Both | 2143 | 139,2 | 0.09 | -2.63 |
| Holden, 1972 | England | F | 234 | 107 | 0.13 | -0.60 |
| | | M | 206 | 85.6 | 0.21 | -0.60 |

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