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Age estimation of the squaliform shark *Centrophorus squamosus* (Bonnaterre, 1788) using the second dorsal spine.

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ABSTRACT

Centrophorus squamosus is one of the target species of the deepwater fishery in the Northeast Atlantic. There is no published information on age for this species. The second dorsal spines of *C. squamosus* were used to produce age estimates. Samples were collected by trawl and longline surveys (1995-1997) and from landings (1997-1998). Marks were observed on the spine of *Centrophorus squamosus* in two separate regions; in the dentine, in cross-sections, and on the mantle. Initial age estimates ranged from 9-35 years. A distinct mark, observed on the distal portion of the stem of *C. squamosus* may be associated with parturition. There is an absence of smaller individuals from the sample. Centra of these species were difficult to section and did not display any banding pattern after staining. The initial age studies, though not validated, indicate slow rates of growth. Length frequencies based on the aged sample show a marked absence of specimens below 82 cm

Keywords; *Centrophorus squamosus*, age, second dorsal spine, length frequency.

INTRODUCTION

Deepwater sharks are the subject of considerable fishing pressure with landings in ICES areas VI and VII reported as 6942 tonnes, (Anon, 1998). Two species are commercially exploited, *Centrophorus squamosus* and *Centroscymnus coelolepis*, carcasses and sometimes livers, rich in squalene, are retained. Discarding may be an important factor in this fishery. One study showed that about 53% of trawl caught discards in the Rockall trough were sharks. While discarding was much lower for longline fishing, sharks accounted for the greatest part (Connolly and Kelly, 1996). Sharks are likely to be highly susceptible to fishing pressure owing to their alleged slow growth, relatively late maturity and low fecundity. *Centrophorus squamosus* may be particularly vulnerable to trawling as the depth range for this species in the Rockall Trough has been reported as between 458 m and 1019 m, with fishing effort concentrated in the upper 1000 m (Gordon and Swan, 1997).

There is little published work on the ageing of deepwater squaliform sharks, and none for the Northeast Atlantic area. Ageing studies of squaliform sharks have concentrated on *Squalus acanthias*, the subject of several ageing studies using dorsal spines; Kaganovskaia (1933) for the north-west Pacific, Bonham et al. (1949) for the north-east Pacific, Holden and Meadows (1962) for the north-east Atlantic, Ketchen (1975) for British Columbia waters, for the western North-Atlantic, (Soldat, 1982; Nammack et al., 1985) and Polat and Gumus (1995) for the Black Sea. Validation of age estimates for spurdog spines have been conducted by (Tucker, 1985; Beamish and McFarlane, 1985; McFarlane and Beamish, 1987). The structure of the spine of the deep-water squaliform species *Centrophorus acus* was described by Tanaka (1990), along with its usefulness for obtaining age estimates.

The current study aims to describe the basic structure of the 2nd dorsal spine of *Centrophorus squamosus*, and the use of this organ for ageing studies of the species. This paper represents the first attempt to estimate age of a deepwater squaliform shark species from the Rockall Trough.

MATERIALS AND METHODS

Sharks were obtained during the deepwater sampling surveys undertaken by the Marine Institute between 1995 and 1997. In total 3 trawl and two longline surveys were completed during this period (Connolly and Kelly, 1997; Kelly et al., 1997; Connolly and Kelly, 1997; Kelly et al., 1998). Fishing was carried out in 5 separate areas of the Rockall Trough west of Ireland and Scotland, see Figure 1. Samples of sharks were also obtained from deepwater commercial trawlers.

Total length (cm), taken as the length from the snout tip to the posterior tip of the caudal fin – depressed along the anterior-posterior axis of the fish – was measured to the nearest centimetre below the actual length of the fish. Weight (g) and sex were recorded. Spines were removed by cutting towards the notochord. Second dorsal spines were placed in Sterilin[®] tubes and preserved in 70 % alcohol. The entire spine was not always removed as this had a detrimental effect on the marketability of the sharks. From these samples second dorsal spines from 74 females, of total length 89 cm to 137 cm and 25 males, of between 93 and 118 cm taken on the above sampling trips were placed in numbered multiwell plates containing 4 % hypochlorite bleach for 28 hours for cleaning. The spines were washed in running tapwater for 4 – 8 hours and allowed to dry overnight. The surface of 51 of these spines was investigated by means of a Cambridge low power dissecting microscope at 10x magnification and reflected light. All bands on the mantle were counted. These and the remainder of the spines were then sectioned transversely using an Isomet[®] low speed saw with diamond blade. Sections were of 500 μ m thickness. Sections were mounted on glass slides with Histokit[®]. Cross sections were taken, in 3 positions (Figure 2), from the spine at intervals of 2 mm, see Figure 2. The first section being made at a distance of 4.5 mm from the spine tip. In many cases the spine tip was worn away. In such cases the distal end of the spine was cut off to give a flat surface and then 3 sections were made, the first at that point and the others at 2 mm intervals. In a small number of spines (5) further sections were to investigate structure in the more proximal regions.

Cross-sections of the spines were read under transmitted light using a binocular microscope (6 – 40 x magnification). Bands were counted in each of the three separate dentinal layers within the spine.

Vertebral centra were removed from beneath the first dorsal fin by making two incisions along the vertebral column. From each shark 4 or 5 centra were removed and stored in labelled Sterilin tubes in 70 % alcohol. Three whole and 3 sectioned centra (500 μ m thickness on an Isomet[®] low speed saw with diamond blade) were stained using crystal violet (Johnson, 1979) alizarin red (La Marca, 1966) and silver nitrate (Stevens, 1975). Sectioning of the centra was done in the longitudinal plane along the supporting struts or intramedialia. Whole centra were viewed with reflected light under low (6-40 x) magnification, while sections were viewed by means of a compound microscope. After treatment as described the centra did not display any obvious banding patterns and were not considered useful for obtaining age readings. First dorsal spines were also taken but were not used in this study.

RESULTS

External Structure of the spine

The surface of the spine was characterised by the presence of longitudinal ridges along the spine stem, converging at the tip. Only the anterior edge of the spine had a mantle of enamel. This mantle occupied only a narrow band on the anterior margin for most of the length of the spine, but towards the tip it extended around the spine almost to the posterior-lateral corners. The pigmentation of the mantle was black and a banding pattern was apparent. The bands were associated with ridges of dentine beneath the mantle surface. The bands were easily distinguishable in the proximal two thirds of the mantle, while the distal third was often worn, and consequently counts of the bands or ridges difficult. In many cases the spine tip was worn away. The part of the stem embedded in the body of the shark displayed no obvious banding structure, did not have a mantle, nor any pigmentation. In a small number of spines a prominent indentation in the mantle was observed towards the tip. This mark lacked the heavy pigmentation of the other bands. While it was not always possible to count the bands on the mantle, distal to this mark, the number never exceeded 3. Beneath the mantle a layer of thickened dentine was present, (Figure 2). This zone increased in thickness proximally along the anterior margin of the spine. Ridges were visible along the surface of this structure and converged towards the tip. In the most proximal third of the spine this structure occupied about 30% of the spine width. Neither the mantle nor the anterior dentine zone was present in the part of the spine embedded in the body.

Structure of the spine in cross-section

Separate zones were identified in the spine cross-sections; the mantle, anterior dentine zone, spine stem and central cavity (Figure 3). The central cavity contained pulp in the more distal portions with the cavity left by the cartilaginous rod apparent in the more proximal sections. The mantle was black in colour and displayed no banding in cross-section. The anterior dentine zone contained many convoluted tubules, which

contained black pigment. No obvious banding pattern was present in the anterior dentine zone

Within the stem, 3 separate layers of dentine were evident (Figure 3), though in some cases the distinction was less clear due to subdivisions within 1 or more of these zones. The inner dentine zone was thickest in the distal sections of the spine. It decreased in thickness proximally, and disappeared below the spine base. The outer and middle zones were of similar thickness. The outer zone was thickest at the posterior-lateral corners while the middle layer thickened in the posterior and anterior parts of the cross-sections.

Counts of ring number

Figure 4 shows a length frequency distribution for the specimens in the current study. While no mode is apparent for either sex there is a clear tendency for females to attain a greater size. None of the FRC deepwater surveys have caught any *C. squamosus* below 80 cm (Kelly et al., 1997). The ranges of ring counts for each dentine zone in each cross-section are given in Table 1.

Table 1. Ranges of age readings of each dentine zone in each cross-section of 2nd dorsal spine of *Centrophorus squamosus*

Section	Outer Dentine	Middle Dentine	Inner Dentine
I	6-16 n = 14	7-12 n = 14	9-34 n=22
II	3-17 n = 36	5-18 n=36	9-34 n = 64
III	2-18 n = 51	5-19 n = 51	9-35 n = 54

A notable feature the counts of the rings in the inner dentine in each of the cross-sections increased with TL but those of the outer and middle dentine zones did not. The total number of rings was greatest in the second cross section from the tip. Proximal cross-sections generally had lower ring counts. The greatest difference in ring number, between the inner dentine zones of the 2nd and 3rd cross-sections was 10, while the percentages varying by ± 1 and ± 2 both were 8.06 %. The percentage of spines varying by more than 2 was 17.74 %. Also noteworthy is the absence of all length classes below 89 cm and 93 cm for females and males respectively. Boxplots of age estimates, based on the counts of ring number from the inner dentine zone are shown for males and females in Figures 6 and 7 respectively. A plot of age readings for both sexes was constructed, based on counts of banding on the mantle, see Figure 5.

DISCUSSION

The structure of the spine of *Centrophorus squamosus* as described above is very similar to that described by for the related species *Centrophorus acus* (Tanaka, 1990). As such it is different, in a number of respects, from the structure of the spine of *Squalus acnathias* as described by Beamish and McFarlane (1985). One of the main differences is the much reduced mantle. Only in the most distal portions of the spine does it cover most of the anterior-lateral faces. Though much reduced in extent in this species the mantle does display a banding pattern. This pattern is fainter than that shown by Beamish and McFarlane (1985), but is similar in that the bands are associated with ridges of dentine on the surface of the spine. The thickened anterior dentine zone described above was also a feature of the spine of *Centrophorus acus* (Tanaka, 1990).

The indentation in the mantle, found in the tip of many of the spines may be associated with parturition. (Holden and Meadows, 1962) describe a white, pigment free zone near the tip of the spines in their study, calling it a birth ring, and noted that spines were often bent at this point. Since no gravid females or post partum juveniles have ever been found in the Rockall Trough by the FRC surveys (Kelly et al., 1998) it is not possible to compare the distance of these marks from the spine tip with the external spine length for pups. The presence of 2 or 3 bands in the portion of the spine distal to this indentation may be an indication of in utero deposition growth. This could suggest that the gestation period be as long as 3 years.

Band counts from the mantle did not show any increase with TL. This is may be due to the increased wear associated with spines of larger specimens. In order to correct for wear of spines, Ketchen (1975) derived a function of spine base diameter and age count for unworn spines. This was used to obtain conceptual ages for worn spines by adding the age obtained from the function, based on the spine diameter at the most distal point without wear. This method was followed by Polat and Gumus (1995) and Nammack et al. (1985). The correction method requires a large number of unworn spines in order to derive the relationship and may be unsuitable for the present study where few spines did not exhibit some signs of wear. The banding pattern evident on the mantle of the spines may be analogous to that on the mantle of the spine of *S. acanthias*, shown to be annual by Beamish and McFarlane (1985) in their validation study. This study, updated in McFarlane and Beamish (1987) concludes that these marks are the most useful for ageing of this species. Very few of the spines under study were not worn to the extent that all growth bands could be read. The counts were, however, in the same range as counts of rings in the inner dentine. This may suggest that the inner dentine rings are a reflection of growth. Validation of age estimates should show that the fish in question is not older or younger than estimated and the the annulus is in fact annual [Beamish, #58], though this is likely to prove expensive and logistically difficult to carry out in the deepwater environment of the Rockall Trough.

Rings were observed in all 3 dentine zones in cross-sections of the spine stem. However, only those in the inner zone increased with length. The thickening of the inner dentine zone towards the spine tip suggests that the perichondrium continues to invest in the stem at the central cavity. This thickening of the inner dentine has been reported by Tanaka (1990) for *C. acus* and by Beamish and McFarlane (1985) who

found oxytetracycline had been deposited in this zone for *S. acanthias*. Boxplots of preliminary age estimates based on ring count in the inner dentine for males and females, suggest growth to be slow. Holden and Meadows (1962) and Soldat (1982) took cross-sections of the spine and described 3 dentinal zones in the stem of *S. acanthias*. Both works describe ring count decreasing for sections taken too close to the spine tip. This was found in the case of *C. squamosus* in the current study. Subsequent years of growth were not represented in the oldest part of the spine – the most distal part. In the present study the ring count in the inner dentine increased to a maximum at the second cross-section which approximated to the apex of the internal cavity. Beamish and McFarlane, (1985) demonstrated that of the banding on the mantle of the spine of *S. acanthias* was not an external expression of the rings seen in cross-section. Banding in the mantle was due to an asynchrony between the upward growth of the stem and the deposition of the mantle by the enamel gland. Slow growth of the spine stem resulting in ridges of enamel, deposition of which continued at a constant rate.

No published work on the use of vertebral centra for ageing of *C. squamosus* exists. The brittle nature of the intermedialia and the focus, both of which are composed of poorly calcified cartilage which are unlikely to stain using a calcium specific stains like alizarin red or silver nitrate. Soldat (1982) and Polat and Gumus, (1995) used the silver nitrate treatment of centra of *S. acanthias*, but did not obtain any clear banding patterns either. The only published ageing study of a squaliform shark to concentrate on centra was by Jones and Geen (1977), who used X-ray spectrometric analysis of vertebrae of *S. acanthias*.

Age readings were taken from the mantle and from the inner dentine zone. The readings from the mantle showed no increase with length. This may be explained by wear, since few of the spines were not worn to some extent. The ring counts in the inner dentine were greatest in the second cross-section. If growth proceeds in the same manner in this species as has been described in *S. acanthias* (Beamish and McFarlane, 1985) then rings in the inner dentine may be used to obtain age estimates. However, validation of such age estimates is required.

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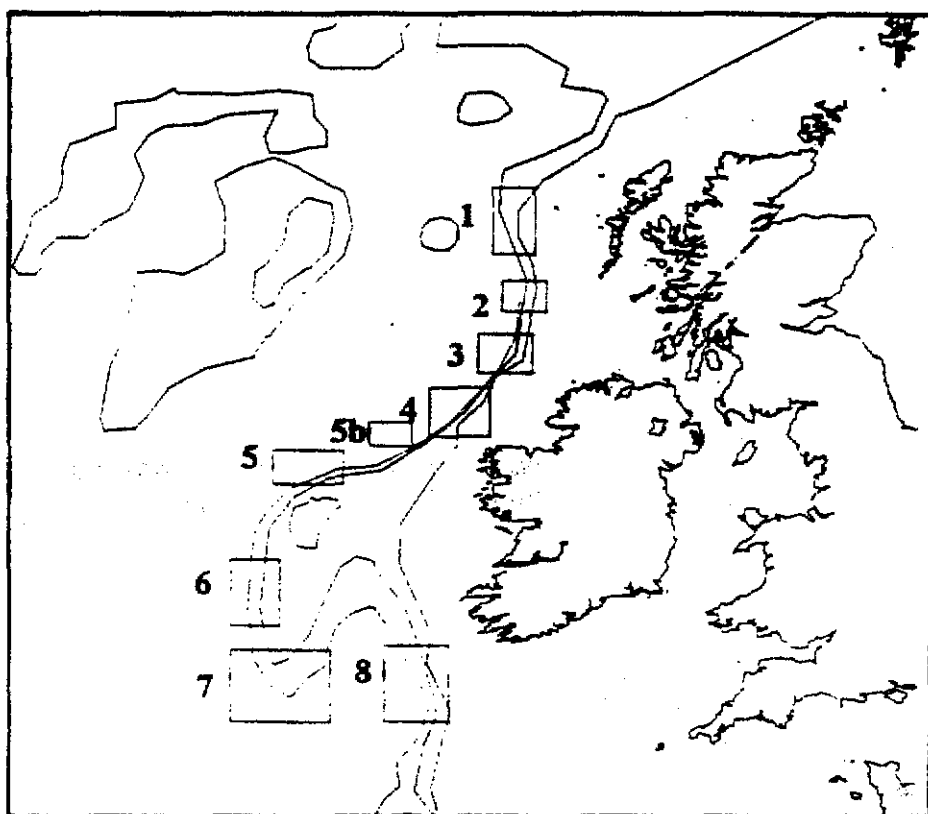


Fig. 1.4 Map showing area fished during deepwater surveys

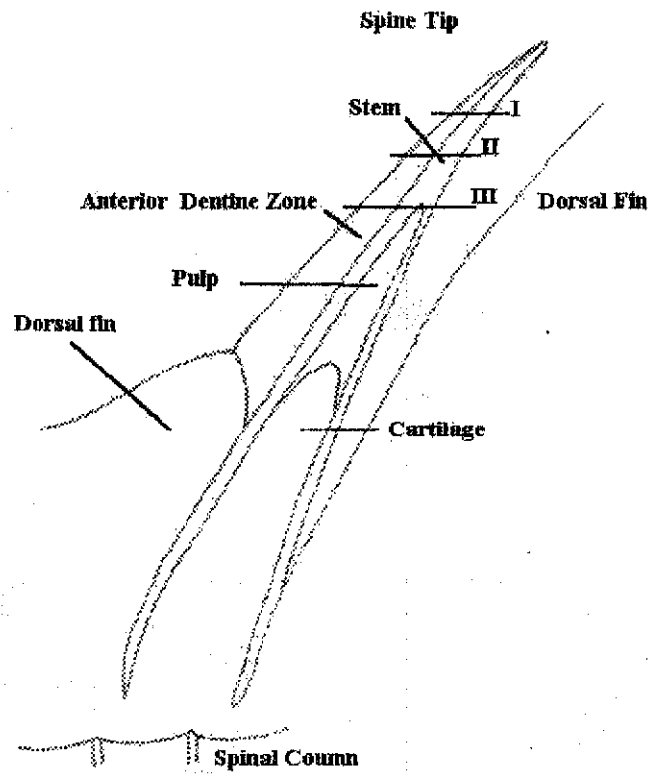


Figure 2. Diagram of the structure of *Centrophorus squamosus* second dorsal spine, Roman numerals denote approximate positions of cross-sections made.

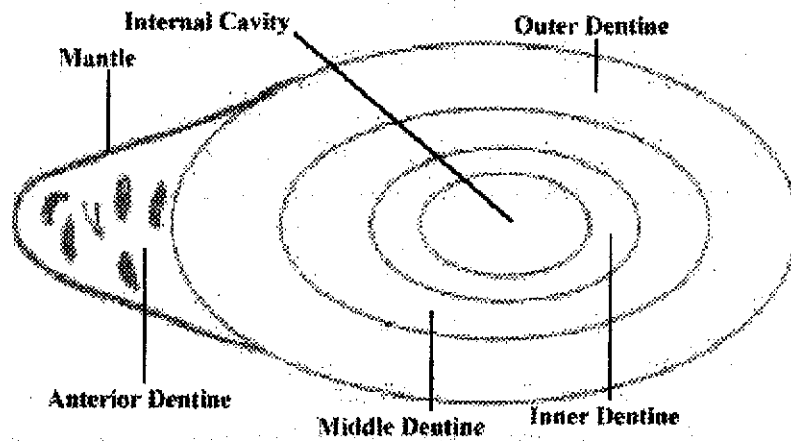


Figure 3. Diagram of cross-section of second dorsal spine of *Centrophorus squamosus*.

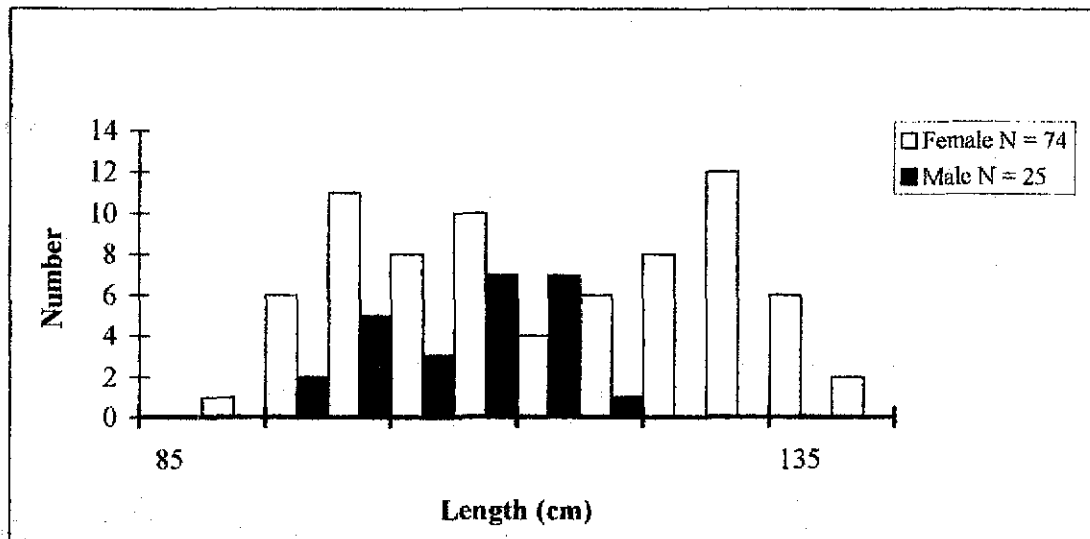


Figure 4. Length frequency distribution for *Centrophorus squamosus* used for age estimation in the current study.

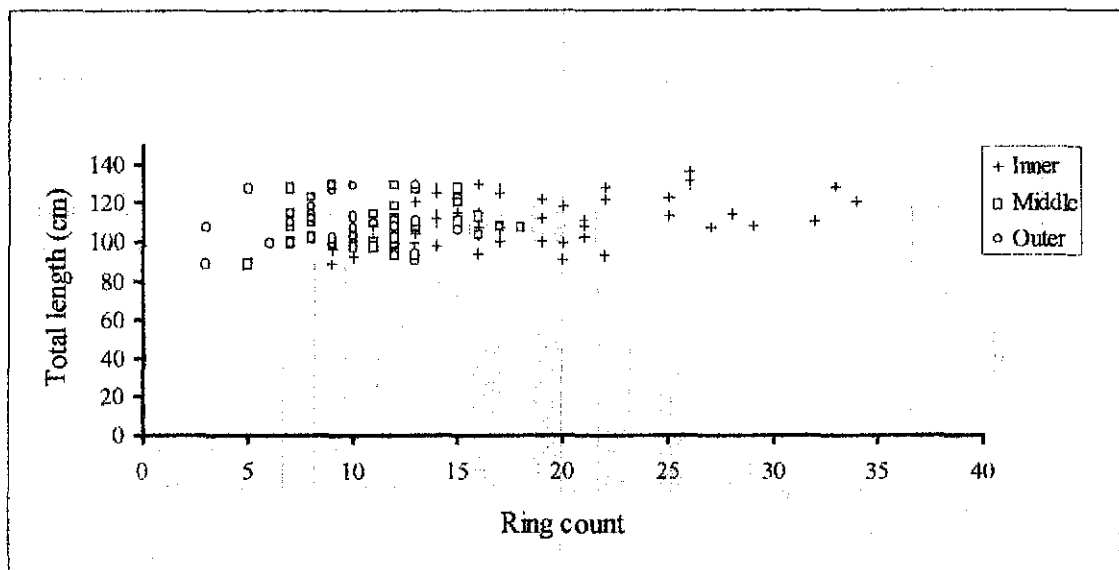


Figure 5. Results of counts of rings in each of the 3 dentine zones in 2nd cross-section of *Centrophorus squamosus*.

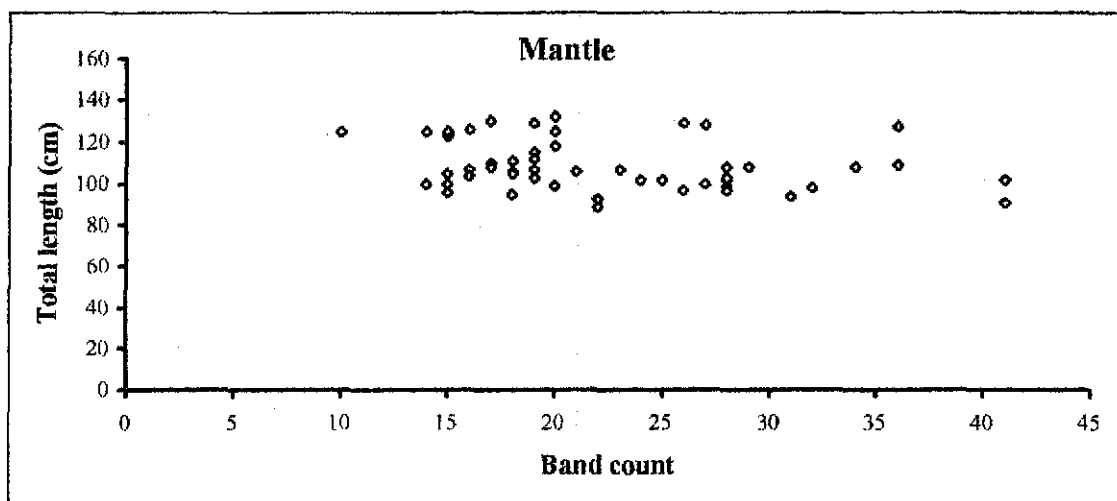


Figure 5. Band counts from mantle with total length (cm).

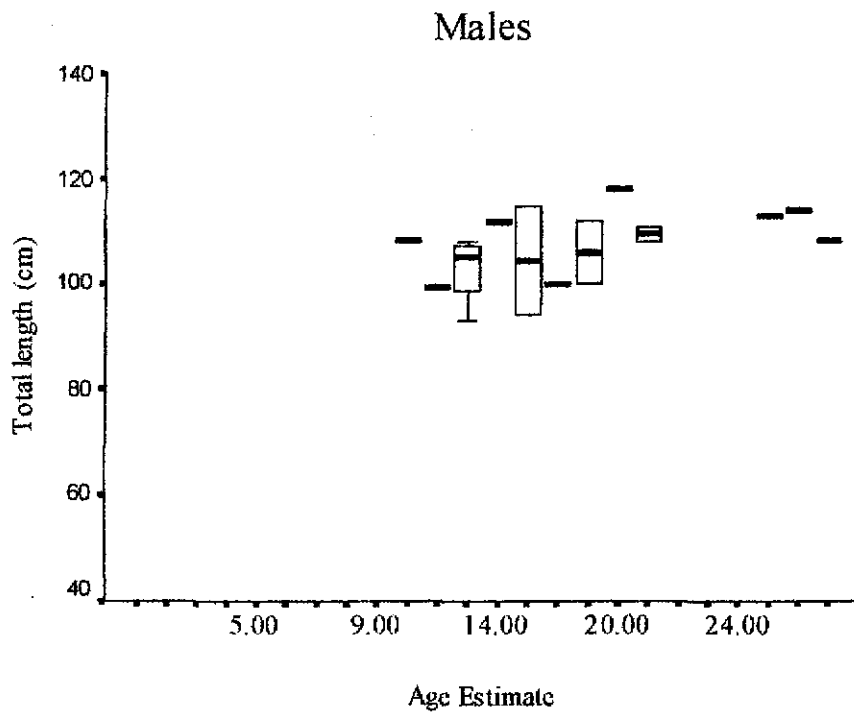


Figure 6. Boxplot of age estimates by length, based upon ring counts in inner dentine of II cross-sections of *Centrophorus squamosus* males N = 25.

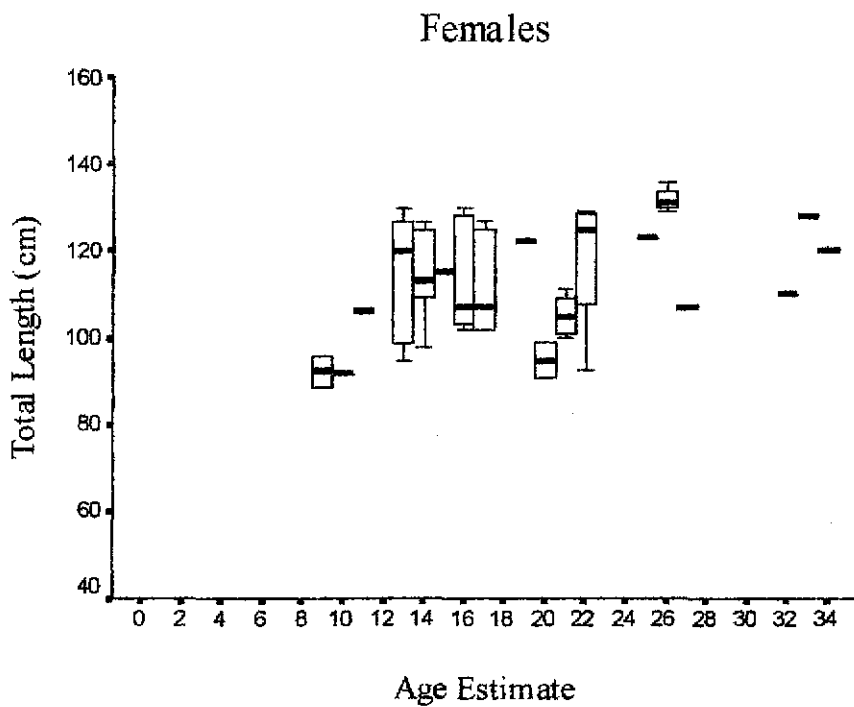


Figure 7. Boxplot of age estimates by length, based upon ring counts in inner dentine of II cross-sections of *Centrophorus squamosus* females N = 74.

