# Annulus Development on the Second Dorsal Spine of the Spiny Dogfish (*Squalus acanthias*) and Its Validity for Age Determination

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Annual marks were visible in three different areas of the dogfish (*Squalus acanthias*) spine. The annuli in the mantle, the stem base, and the inner dentine develop independently of each other. Annuli that formed on the mantle were readily identifiable externally, making them the most useful for age determination. The mantle annulus is an accumulation of pigment that appears to form because enamel deposition is not synchronous with the upward growth of the spine, resulting in the production of darkened bands that often form ridges. We validated our interpretation of annuli from fish aged 20–70 yr by tagging and recovering dogfish that were injected with oxytetracycline. Through validation we were able to demonstrate that some previous studies have underestimated age, resulting in a misunderstanding of important life history parameters.

On a observé des marques annuelles dans trois régions différentes de l'épine de l'aiguillat commun (*Squalus acanthias*). Les anneaux au niveau du manteau, de la racine et de la couche intérieure de dentine se développent indépendamment les uns des autres. Les anneaux qui se sont formés au niveau du manteau étaient facilement identifiables de l'extérieur, ce qui en fait les éléments les plus utiles pour la détermination de l'âge. L'annulus du manteau correspond à un accumulation de pigment qui semble se former du fait que le dépôt d'émail ne se produit pas en même temps la croissance vers le haut de l'épine. Cela entraîne la production de bandes foncées qui forment souvent des crêtes. Nous avons prouvé la justesse de l'interprétation que nous avons faite des anneaux à partir de poissons dont l'âge variait de 20 à 70 ans en étiquetant et en recapturant des aiguillats auxquels on avait injecté de l'oxytétracycline. Nous avons pu ainsi démontrer que l'on avait, dans certaines études antérieures, sous-estimé l'âge, donnant ainsi lieu à une interprétation erronée de paramètres importants du cycle vital.

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Effective management of spiny dogfish (*Squalus acanthias*) off Canada's west coast requires an accurate method of age determination. Dorsal spines have been used by Kaganovskaia (1933), Bonham et al. (1949), Aasen (1961), Holden and Meadows (1962), Ketchen (1975), and Soldat (1982). While some attempts were made at validation, the method has not been validated using a quantitative technique that is applicable for all age groups (Beamish and McFarlane 1983). In this study we tagged and injected fish with oxytetracycline (OTC) to mark the spines. The recovery of spines from these tagged and injected fish provides a quantitative method of validation. In addition, we studied the development and growth of the spine to determine the method of annulus formation. Markert (1896) and Ritter (1900) described the histology of the spine but did not relate it to spine growth or age determination. Holden and Meadows (1962) have discussed spine growth in relation to age determination but their description of formation of annuli is not in agreement with its histology. Therefore, we reexamined the histology of the spine and the theory of Holden and Meadows (1962) to resolve the question of how an annulus is formed.

## Materials and Methods

The second dorsal spine was used in all studies. Spines were removed by cutting horizontally just above the notochord to ensure that the spine base and stem were intact. Spines were cleaned and air-dried in 14.5-kg ungummed Kraft envelopes. Surface annuli were determined using a dissecting microscope. All ages were estimated by a reader experienced in the method outlined by Kaganovskaia (1933), Bonham et al. (1949), Aasen (1961), Holden and Meadows (1962), and Ketchen (1975). The modifications outlined by Ketchen (1975) were used except that all ages were calculated from birth. Most dogfish are born late in the year (Ketchen 1972). The birthdate was assumed to be January 1; however, the age of one was assigned to fish in the second year after birth, when they were 12–14 mo old.

Approximately 400 spines ranging in age from 0 to 4 yr were collected during research cruises in 1978 for measurement of total length (base to tip) and mantle length (measured from ventral anterior base of the mantle to tip). The distance between annuli was measured from a subsample of 20 spines from each of the 2-, 3-, and 4-yr age groups. Cross sections of dried spines were made by embedding the spine in epoxy and sectioning according to the procedure outlined by Beamish (1979) and Chilton and Beamish (1982).

Longitudinal sections were cut from spines from two fish of age 4 and one each of age 10, 16, and 24. The enamel and pigment were ground away with sandpaper; the exposed surface was then examined according to the procedure of Holden and Meadows (1962).

Fifteen spines collected from younger fish from the Strait of Georgia in 1978 were preserved in Bouins for 48 h and transferred to 70% ethanol and subsequently embedded in paraffin. Histological sections were stained with alizarin red S.

Dogfish from the Strait of Georgia were tagged and given an injection of OTC at the rate of approximately 25 mg/kg body weight (McFarlane and Beamish 1985). The injection procedure was similar to that used for sablefish (*Anoplopoma fimbria*) (Beamish et al. 1983). This dosage was selected because dosages of 75 mg/kg and higher cause mortalities in sablefish (Beamish et al. 1983), and preliminary studies indicated that an acceptable mark was produced at a rate of 25 mg/kg body weight. Spines from injected fish that had been at liberty for 2 yr or more were examined for age validation. Spines from injected fish were processed and examined according to the procedure of Chilton and Beamish (1982). After locating the OTC mark, the distance from the base of the mantle on the anterior edge of the spine to the OTC mark was measured to the nearest 0.1 mm. Under reflected light the position of the mark was located using this measurement and the number of annuli that formed after the mark was counted.

## Results

### Description of the Spine

The spine is triangular in section and consists of three major structural components; the cartilaginous interior, the stem, and the mantle (Fig. 1). The interior of the spine base is filled with cartilage and surrounded by pulp tissue. The cartilage and pulp tissue degenerate towards the spine tip leaving a central cavity. The cartilage that supports the base of the spine is also continuous with the cartilaginous support for the dorsal fin. The stem surrounds the pulp tissue and is the main body of the spine. The stem consists of three layers of dentine: inner, middle, and outer. The mantle originates at the enamel organ and covers the two anterior–lateral faces of the spine distal to this organ. The midportion of the posterior surface of the spine is not covered by the mantle. The mantle consists of an inner dentine layer, a midportion containing pigment, and an outer layer of enamel.

Cartilage is produced along the ventral and anterior–lateral surfaces of the spine base. The perichondrium (Fig. 1) is visible along the interior of these surfaces. Cartilaginous cells or chondrocytes are distributed within this homogeneous tissue. Towards the tip of the spine the cartilage becomes more cellular and eventually these cells die.

The stem originates close to the ventral surface of the spine base. The anterior–lateral surfaces develop closer to the base than the posterior surface. Odontoblasts at the base of the stem produce an intercellular matrix of collagen fibres and amorphous cementing material. The cementing material is deposited by processes of the odontoblasts into the collagen fibre matrix producing the dentine layer. This layer becomes denser in the centre as it thickens distally. As more dentine is deposited the odontoblasts are displaced progressively further away and the dentinal tubules, formed by the processes of the odontoblasts, become elongated. Dentine is produced on the internal and external surfaces of the stem increasing the thickness and forming an outer, middle, and inner dentine layer. The middle and outer layers are uniform, while the inner layer increases in width from the base to the tip. The anterior–lateral sides of the stem are much thicker than the posterior side. The inner and outer layers are penetrated by dentinal tubules. Upward growth results from the continuous deposition of dentine at the base of the stem, while outward growth is a consequence of cartilage production in the centre of the spine.

A pulp cavity, within the spine, containing nerves and blood vessels is situated between the perichondrium and stem odontoblasts.

The mantle originates at the enamel gland and consists of an outer layer of enamel, a midlayer of pigment, and an inner layer of dentine. Enamel formation is similar to that of dentine in that it involves secretion of an intercellular matrix, followed by calcification of that matrix. The production of pigment and mantle dentine occurs adjacent to the inner surface of the enamel organ. The odontoblasts near the organ produce thin layers of dentine. Pigment (melanin) is produced by the melanophores at the inner surface of the organ and is deposited between the enamel and the dentine. This mantle is laid down on the stem dentine. As this new layer passes the top of the enamel organ, enamel is added from the enamel epithelium (ameloblasts) at the opening of the organ to complete the mantle. As the mantle dentine is laid down, cell-filled canals are formed that make up the marginal canal system, the purpose of which is uncertain although the cells could be responsible for calcification of the dentine.

Darkly pigmented ridges appear on the surface of the mantle. These ridges are used to estimate the age of dogfish. While the method of age determination has been described as counting darkened bands (Holden and Meadows 1962; Ketchen 1975), we define the annulus to be a darkened band or ridge or both. In most cases the ridges are darkened; however, occasionally a ridge does form that is not darkened.

When smoothing the ridges from five spines using sandpaper, we observed that the ridge could be removed from all spines without disturbing the pigment layer. The pigment layer could be removed without substantially disturbing the dentine layer. This indicates that the ridges consisted of thickened enamel.

We believe the annulus forms because the mantle formation is not synchronous with the upward growth of the spine. When spine growth is reduced there is a concentration of pigment and a thickening of the enamel.

### Spine Lengths

There was no significant difference between the mean lengths of similar aged fresh and dried spines (Table 1). There also was no difference in the mean lengths of similar aged spines from males and females. Thus, all measurements were made using dried spines and combining males and females. The average growth of the spine during the first, second, third, and fourth years was 0.33, 0.16, 0.04, and 0.25 cm, respectively (Table 1). The length of the nonenameled portion of the stem (total length minus mantle length) ranged from 0.81 to 1.09 cm for this same period (Table 1) or 0.07 cm/yr. Thus, the total spine growth in the first 4 yr was not sufficient to cause dentine formed at the base of the stem to be covered with enamel.

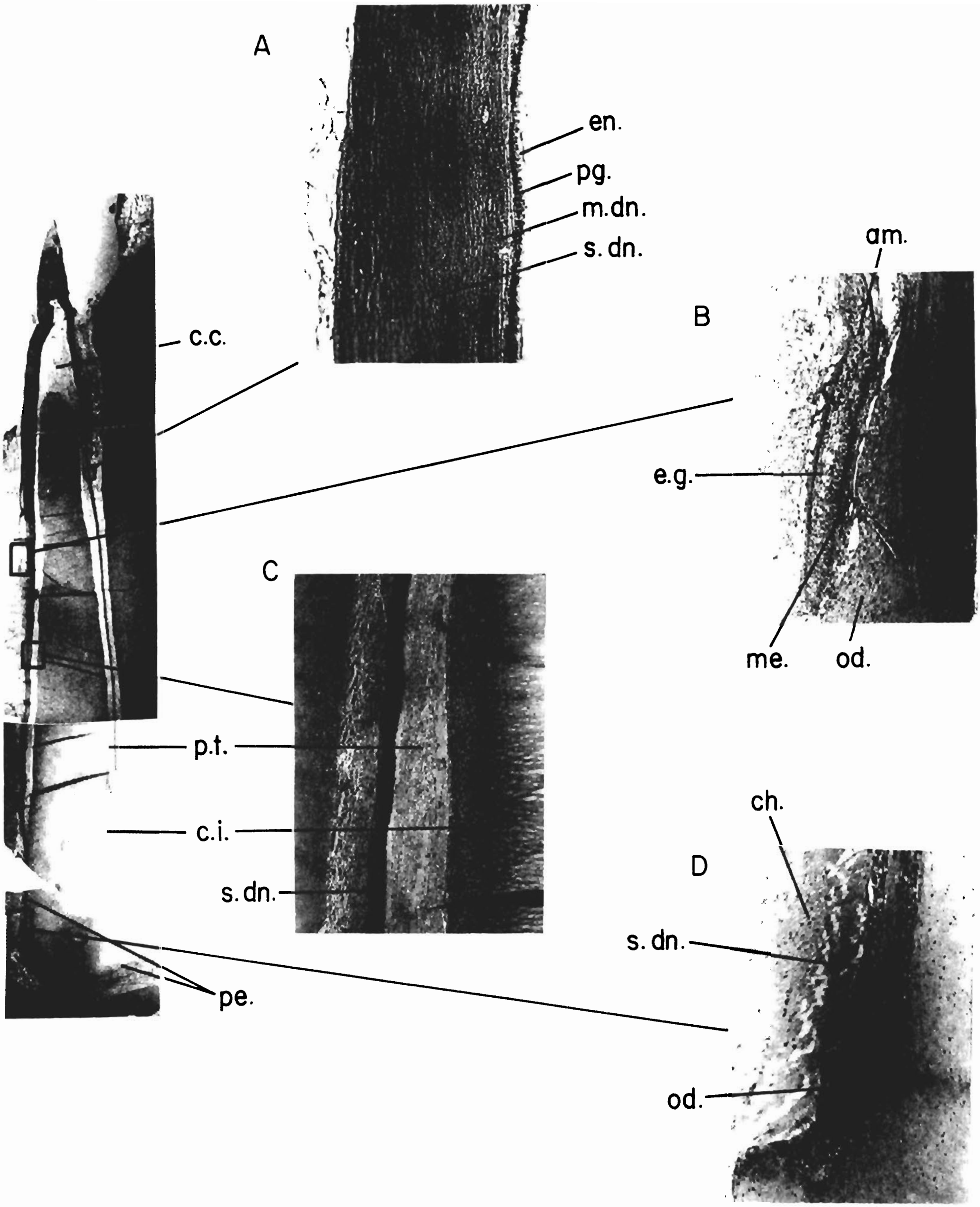


FIG. 1. Longitudinal section through a dogfish spine (extreme left): c.c. = central cavity, p.t. = pulp tissue; c.i. = cartilaginous interior; pe. = perichondrium. (A) Posterior edge: en. = enamel; pg. = pigment; m.dn. = mantle dentine; s.dn. = stem dentine. (B) Enamel gland: e.g. = enamel gland; od. = odontoblasts; me. = melanophores; am. = ameloblasts. (C) Anterior edge of stem. (D) Anterior laterial area of stem formation: ch. = chondrocytes.

TABLE 1. Measurement of fresh and dried dogfish spines for fish aged 0+ to 4+.

| Age | No. of observations | Total spine length (cm) | Mantle length (cm) | Stem base (nonenamelled portion) |
|---|---|---|---|---|
| | | *Dogfish spine lengths, fresh* | | |
| 0 | 3 | 2.02±0.05 | 1.21±0.04 | 0.81±0.01 |
| 1 | 9 | 2.41±0.21 | 1.47±0.17 | 0.94±0.07 |
| 2 | 19 | 2.64±0.17 | 1.62±0.12 | 1.02±0.07 |
| 3 | 30 | 2.68±0.14 | 1.64±0.11 | 1.04±0.06 |
| 4 | 18 | 2.78±0.20 | 1.69±0.16 | 1.09±0.11 |
| Mean annual increment | | 0.19±0.16 | 0.12±0.11 | 0.08±0.06 |
| | | *Dogfish spine lengths, dried* | | |
| 0 | 29 | 2.05±0.30 | 1.25±0.24 | 0.81±0.10 |
| 1 | 47 | 2.38±0.18 | 1.49±0.11 | 0.89±0.10 |
| 2 | 63 | 2.54±0.20 | 1.59±0.10 | 0.96±0.16 |
| 3 | 69 | 2.59±0.19 | 1.62±0.14 | 0.98±0.09 |
| 4 | 58 | 2.84±0.20 | 1.77±0.15 | 1.08±0.09 |
| Mean annual increment | | 0.20±0.12 | 0.13±0.09 | 0.07±0.03 |

### Growth Pattern in the Stem

When the cleaned and dried stem (spine) base is viewed with reflected light there is a pattern of alternating growth zones. When the mantle is scraped off, it is possible to see that this pattern extends beneath the mantle towards the spine tip. These zones are the "cone bases" described by Holden and Meadows (1962). The shape of the growth zones is identical to the shape of the spine base, suggesting that they form as a consequence of growth changes at the spine base. Injected fish also had an OTC mark at the spine base (Fig. 2A). The number of zones that formed ventral to this mark on the 20 specimens examined was equal to growth periods at liberty, indicating that these marks were formed annually. However, these stem annuli had a different shape than the mantle annuli and clearly were not formed in the same manner.

Cross sections from 12 spines revealed growth checks only in the inner dentine. They were difficult to count, being separated from each other by a number of less obvious rings. At the outer edges these checks become closely spaced, irregular, and difficult to define, particularly in fish older than 10 yr. The outer dentine shows only a few obscure rings. Cross sections of five spines from fish injected with OTC did have an OTC mark in the inner dentine, indicating that at this time, growth in the inner dentine appears synchronous with growth at the spine base and the mantle.

### Validation

As of December 31, 1983, 13 826 fish were injected with OTC and released. A total of 369 fish have been recaptured of which the spine was recovered from 261 fish. Sixty-six percent had an OTC mark. For this validation study we used fish that had been recovered after being at liberty for 2 yr or more. Fish that were at liberty less than 2 yr were excluded because the slow growth of the spine made it difficult to separate the OTC mark from the annulus. Included in this report is one fish recaptured in 1984 after 4 yr at liberty and one fish recaptured in January 1985 after 5 yr at liberty (Fig. 3A, 3B).

There were 11 fish that had been at liberty for 2 yr, five fish for 3 yr, one fish for 4 yr, and one fish for 5 yr. Those fish that had been at liberty for 2 yr had developed two annuli below the mark, and four fish at liberty 3 yr had three annuli below the mark (one fish had two annuli below the mark), and the single individuals recovered after 4 and 5 yr had developed four and five annuli, respectively (Table 2). In only one case was there a disagreement with the years at liberty (growth periods) and the number of annuli.

There was a bias in all readings resulting from a knowledge that injected fish could have only been at liberty for a few years. However, in these samples the identification of annuli was not confused by checks or false annuli and was relatively uncomplicated.

To test this bias, all annuli on the mantle of the spine were counted. The spine was reexamined using UV light and the number of annuli that formed prior to tagging and injection (distal to OTC mark) were counted. The years at liberty were then determined by subtracting this latter estimate from the former. In all cases the years at liberty were estimated correctly.

In most of the samples the annulus was easily identified as a ridge that was darker than the areas above and below it. In some older fish, the annuli that formed after the OTC mark were quite closely spaced (Fig. 2B). Under reflected light the annuli were very distinct, forming prominent darkened ridges. In younger fish the annuli tended to be more widely spaced and ridges were less prominent.

Fish that had been at liberty for approximately 2 yr or more ranged in age from 20 to 70 yr (Table 2) and in length from 61 to 111 cm. The tip of the spine showed some wear after an average age of 8–10 yr. Ages older than this were estimated using the relationship between the width, the no-wear point, and spine growth (Ketchen 1975).

The OTC mark frequently appears as a double line (Fig. 2B, 3A, 3B). Since the spine growth is slow and OTC remains active for only a short period of time (Milch et al. 1958), the two lines must have developed simultaneously. The formation of two lines confirms that mantle dentine forms first and subsequently is covered with enamel. This means that there are distinct and separate sites for mantle dentine and enamel formation.

## Discussion

Problems associated with not validating age determinations have been discussed by Beamish and McFarlane (1983). In the absence of a properly validated method for aging dogfish, investigations have grouped growth zones on the spine (Holden and Meadows 1962), or rejected difficult to age spines (Bonham et al. 1949; Holden and Meadows 1962; Ketchen 1975). Unfortunately, in the absence of validation there is no scientific basis for ignoring, grouping, or eliminating material where growth zones are visible. Where this has been done (Bonham et al. 1949; Ketchen 1975), it appears that younger ages may have been produced or there has been a selection for faster growing fish which frequently lay down clearer annuli.

Several studies have attempted to validate this method. While Holden and Meadows (1962) stated that the bands they counted were "proved to be annuli," they presented no substantiation. Ketchen (1975) used a variety of techniques, including length frequency analysis and mercury accumulation. Again, these techniques cannot validate an age determination method over the age range of the species. In addition, we have examined length frequencies from a number of locations, and because of

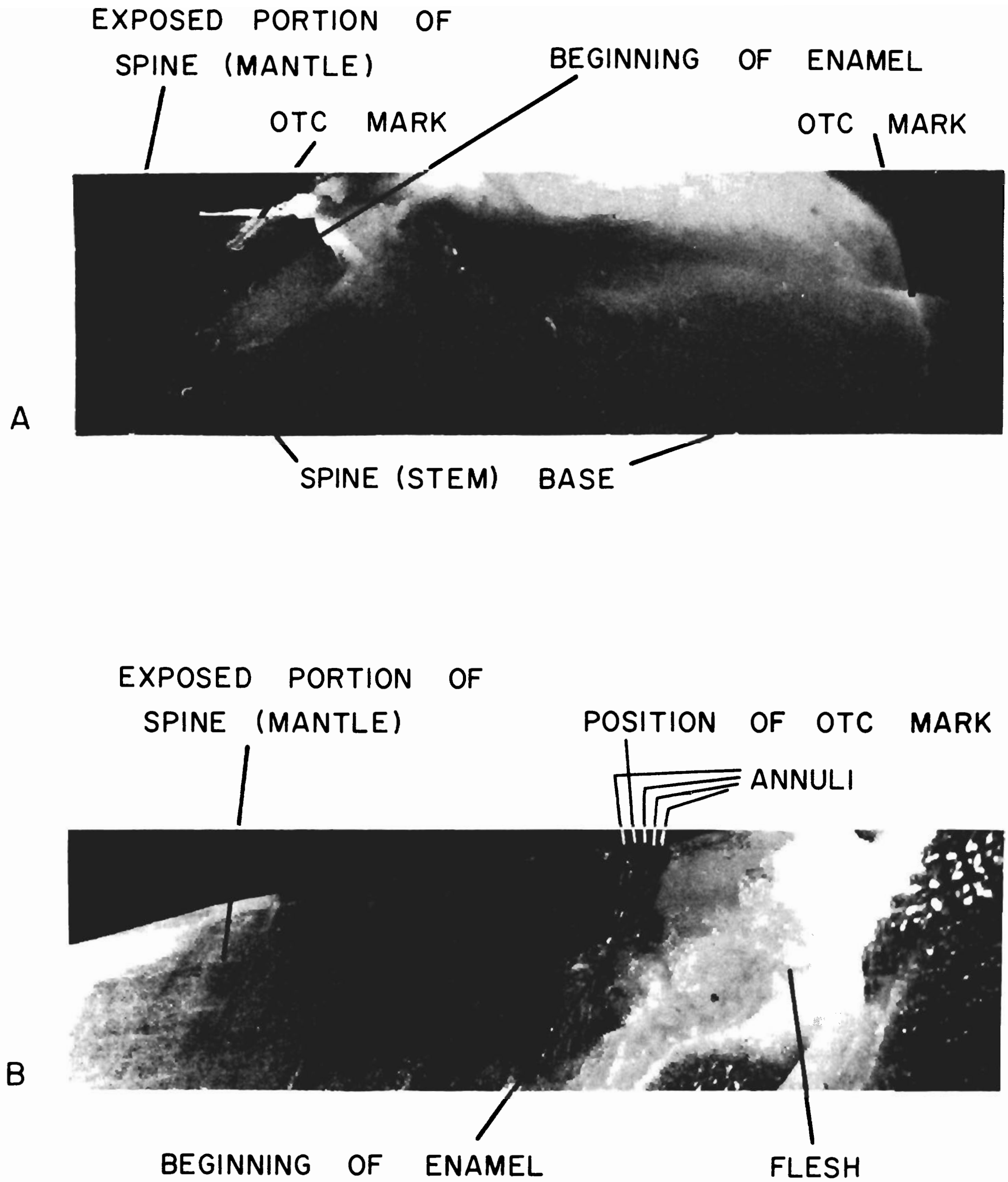


FIG. 2. Dorsal spines from recaptured tagged and injected dogfish. (A) Spine from a 75-cm male recaptured in October 1983 after 2 yr at liberty showing that the OTC mark forms both in the mantle (double line) and the stem dentine. (B) Spine from a 111-cm female recaptured in November 1983 after 3 yr at liberty showing slow growth as indicated by the spacing between annuli.

the slow growth rate it is difficult to identify age groups (Beamish et al. 1982). Jones and Geen (1977) used X-ray spectrometry to show that concentrations of calcium and phosphorus in vertebrae corresponded to the annulus. However, this does not prove that the growth zone or the concentration of the minerals occurs annually.

In our study the placement of a "OTC mark" and subsequent recapture of individuals does allow examination of the growth zones that form during the period at liberty. Because the annulus did develop once during the year for fish aged 20–70 yr, we have demonstrated that the growth zones on the second dorsal spine can be used for age determination. We concluded that all darkened bands, ridges, or both must be counted. In most cases the ridge was also darkened. In addition an annulus could be identified on the stem. This annulus was visible on dried specimens in the base of the spine and was visible when the mantle was removed. However, because of the difficulty in removing the mantle, we recommend that growth zones on the mantle be used for age determination.

Our description of the histology of the spine was identical to the descriptions of Markert (1896) and Ritter (1900). However, our description of the growth of the spine and annulus formation does not agree with those of Holden and Meadows (1962) and Soldat (1982). Holden and Meadows (1962) stated that the stem

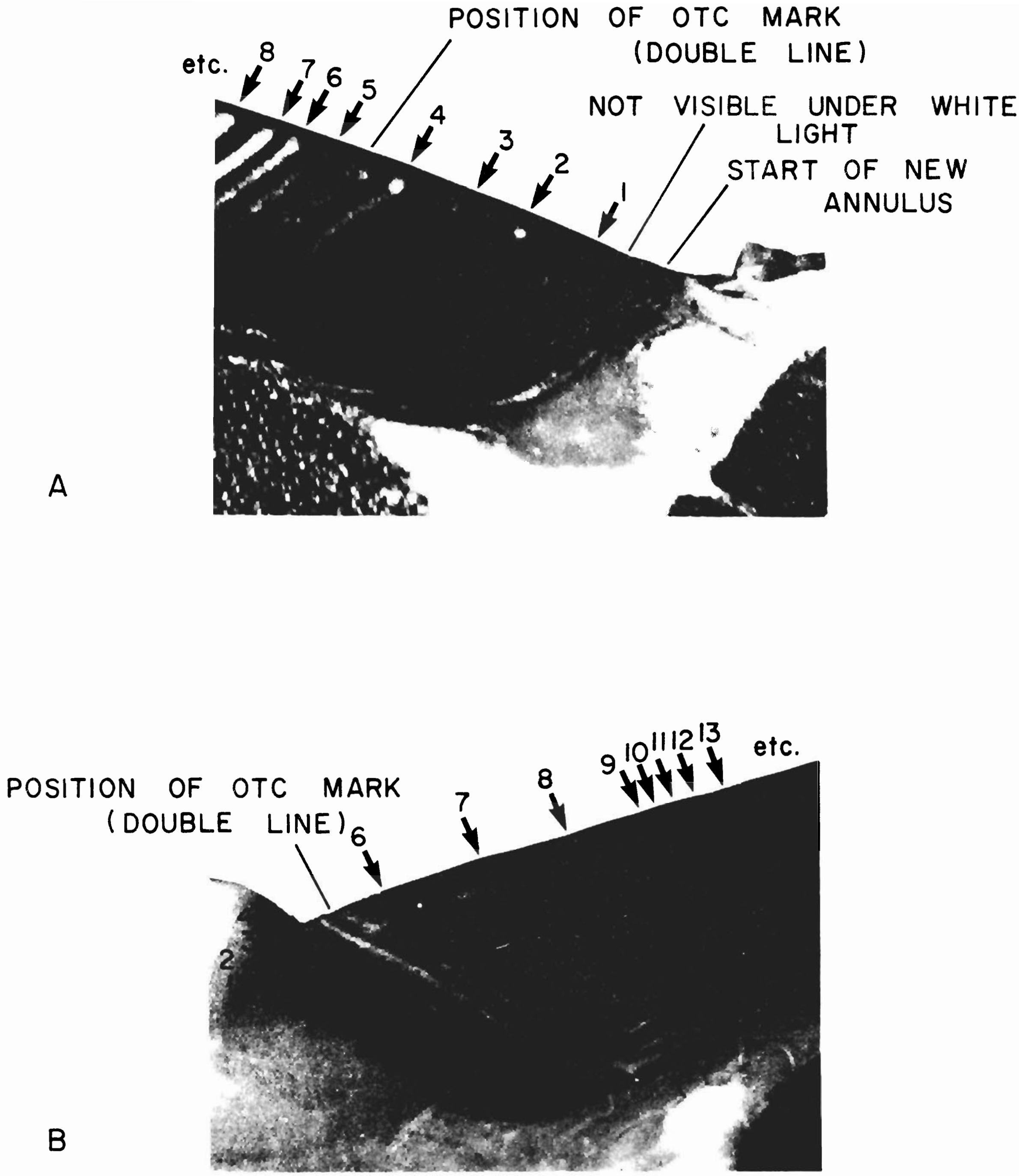


FIG. 3. (A) Spine from a 75-cm male recaptured in late 1984 after 4 yr at liberty. Photographed using UV light. (B) Spine from a 91-cm female recaptured in January 1985 after 5 yr at liberty showing variable spacing between annuli. The annuli that formed after the fish was injected are closely spaced and could be incorrectly grouped. Photographed using UV light.

annulus was "reflected in the external surface by bands of pigment associated with ridges of enamel." They assumed that the spine grew in a series of overlapping cones with the point of overlap being the ridge.

Our study clearly showed that the mantle annulus was formed independently of the stem annulus. Stem annuli result from differential growth at the base of the spine and slowly move away from the base. A number of these annuli may be incorporated in both the unexposed and exposed portions of the spine. Beneath the mantle these growth zones are different in width and shape than mantle growth zones, and chronologically are younger. Since the pattern is produced at the base of the spine, there are no overlapping cones, but rather it is a discontinuous growth pattern with the stem.

The mantle annuli form as a result of a lack of synchrony between the upward growth of the stem and the mantle formation. During periods of slow upward growth it appears that mantle growth is not correspondingly reduced, resulting in a buildup of enamel and a concentration of pigment producing a darkened ridge.

Holden and Meadows (1962) showed that alternating growth zones could be observed in cross sections of the inner dentine.

TABLE 2. Estimated number of annuli that formed on spines from fish tagged and injected with OTC that had been at liberty for 2 yr or more.

| Expected no. of annuli (yr) beyond OTC mark[a] | n | Estimated no. of annuli | Age (yr) at time of recapture[b] |
|---|---|---|---|
| 2 | 11 | 2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2 | 20, 26, 29, 30, 31, 32, 37, 39, 47, 52 |
| 3 | 5 | 3, 3, 3, 3, 2 | 26, 27, 70 |
| 4 | 1 | 4 | 26 |
| 5 | 1 | 5 | 40 |

[a]Annulus formation was assumed to occur from November to April. A fish that was expected to form two or more annuli had to be at liberty for at least two consecutive November–April periods.

[b]Ages could not be estimated on all spines because portions were damaged.

They felt that this alternating pattern of growth occurred annually. The maximum number of annuli, in cross section, occurred just dorsal to the apex of the pulp cavity. We also observed this pattern of alternating growth zones in the inner dentine, but they were not as prominent as the mantle annuli.

The increase in thickness of the inner dentine as it approaches the apex of the spine indicates that the perichrondrial layer adjacent to the pulp cavity continues to deposit dentine along the internal face of the stem. Spine growth and spine wear eventually result in a loss of annuli. While this is a third process by which annuli can be identified in the spine, this loss of annuli and the difficulty of their interpretation makes this aging method unsuitable.

Annuli were found in the cross sections of the inner dentine, longitudinally in the stem, and as darkened ridges in the mantle. Sections of the inner dentine were unsuitable as previously described. The annuli visible longitudinally in the stem could be used for age determination but it was difficult and time consuming to remove the mantle. Therefore, we conclude that the mantle annuli were the easiest to identify. The procedure of measuring the thickness of the "no-wear point" (Ketchen 1975) appears to be the only method of estimating lost annuli. While this measurement probably introduces an important error, we were unable to find a better method of estimating lost annuli.

Our validation of mantle annuli indicated that there is no basis for the "grouping" of darkened ridges (Fig. 2B, 3B). This means that ages assigned by others may underestimate the true age. Our estimates indicate that dogfish are older, later maturing, and slower growing than previously thought.

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Mike Smith participated in all aspects of this study, which benefitted from his long experience with dogfish. A number of fishermen and staff of the Fisheries Research Branch participated in the tagging program. Students and staff of the Campbell River and Southgate Secondary Schools also assisted in the study and we appreciate the continued cooperation of V. Egan and D. Brown. Histological sections were prepared by John Bagshaw.

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