Surface bands on deepwater squalid dorsal-fin spines: an alternative method for ageing Centroselachus crepidater

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Abstract: Bands on the external surface of the second dorsal-fin spine proved to be a novel method of estimating the age and growth of Centroselachus crepidater. Bands that followed the shape of the spine base were enhanced with an alizarin red derivative. Internal bands in spine cross sections were also examined. The number of both external and internal bands increased with animal size, although most spines had more external than internal bands. External bands were more reliable and were assumed to be annuli. The rate of band formation differed after five bands had been formed, and internal bands ceased forming after 30 years. Females to 54 years old and males to 34 years old were examined. Maturity occurred over a wide age range, with estimates of 20 years for females and 9 years for males. The youngest pregnant female was 27 years old. The Francis reparameterized von Bertalanffy growth model found similar growth for males and females, and the von Bertalanffy equations were $L_t = 96.12(1 - e^{-0.072(t+6.13)})$ for females and $L_t = 73.22(1 - e^{-0.141(t+2.99)})$ for males.

Résumé : L’étude des bandes sur la surface externe de la seconde épine dorsale s’est avérée être une nouvelle méthode d’estimation de l’âge et de la croissance chez Centroselachus crepidater. Nous avons accentué le contraste des bandes qui suivent la forme de la base de l’épine à l’aide d’un dérivé du rouge d’alizarine. Nous avons aussi examiné les bandes internes sur des coupes transversales d’épines. Le nombre de bandes, tant externes qu’internes, augmente en fonction de la taille de l’animal, bien que la plupart des épines portent plus de bandes externes qu’internes. Les bandes externes sont plus fiables et nous considérons qu’il s’agit d’annulus. Le taux de formation des bandes est différent après la formation des cinq premières bandes et la formation de bandes internes cesse au bout de 30 ans. Nous avons examiné des femelles d’âge maximal de 57 ans et des mâles de 34 ans. La maturité est atteinte sur une gamme étendue d’âges et nous estimons l’âge de la maturité à 20 ans chez les femelles et de 9 ans chez les mâles. La femelle gravide la plus jeune avait 27 ans. Le modèle de croissance de von Bertalanffy avec les paramètres déterminés par la méthode de Francis indique une croissance semblable chez les mâles et les femelles; les équations de von Bertalanffy sont $L_t$ des femelles $= 96.12(1 - e^{-0.072(t+6.13)})$ et $L_t$ des mâles $= 73.22(1 - e^{-0.141(t+2.99)})$.

[Traduit par la Rédaction]

Introduction

Deepwater dogfishes are a major bycatch component of many of the world’s demersal fisheries, and several species are directly targeted for the shark fillet and liver oil markets. In Australia, deepwater dogfishes are primarily caught as byproduct to demersal trawl fisheries, and the estimated annual value of deepwater dogfishes exceeds AUD$1.5 million (Daley et al. 2002). In 2005, eight species of deepwater dogfishes, including the golden dogfish, Centroselachus crepidater (Bocage and Capello 1864), became managed under a “basket” quota system, with a total allowable catch set at 200 tonnes (P. Shoulder, Australian Fisheries Management Authority, P.O. Box 7051, Canberra Business Centre, ACT, Australia 2610, personal communication).

Centroselachus crepidater is uniformly dark brown to black, with a slender body, long snout, and small dorsal-fin spines. It occurs on the continental and insular slopes in depths of 270–1300 m in the eastern Atlantic (Iceland to southern Africa), the Indian Ocean (Aldabra Islands and India), the eastern Pacific (northern Chile), and western Pacific and eastern Indian oceans (New Zealand and southern Australia) (Last and Stevens 1994).

Age information forms the basis for the calculations of growth rate, mortality rate, and productivity, making it one of the most influential biological variables for estimating...
population status and assessing the risk associated with exploitation (Ricker 1975; Musick et al. 2000; Campana 2001). Band counts from vertebrae are the most commonly used method for ageing elasmobranchs (Cailliet and Goldman 2004), although most dogfish vertebrae are poorly calcified with no visible banding (Jones and Geen 1977).

Kaganovskaia (1933) first used the second dorsal-fin spine to age the spiny dogfish (*Squalus acanthias*), and the structure and function of dorsal-fin spines has since been documented (Maisey 1979; McFarlane and Beamish 1987). *Squalus* species often have spines with a banding pattern on an enamel cap, and these bands are more commonly used to age dogfishes (Holden and Meadows 1962; Nammack et al. 1985; McFarlane and Beamish 1987). However, most deepwater dogfish spines do not possess an enamel cap, and Tanaka (1990) reported an alternative ageing technique using an internal banding pattern in the spine cross sections (hereafter called “internal bands”). Over the past decade, these internal bands have been used to age several deepwater dogfishes (e.g., Tanaka 1990; Guallart Furio 1998; Clarke et al. 2002b). However, most studies report the usefulness of spines as a tool for ageing rather than estimating growth, and the validation of deepwater dogfish age estimates has never been attempted.

Holden and Meadows (1962) were the first to note the growth bands on the stem of the *S. acanthias* spine. In this species, each band followed the shape of the spine base and could only be counted after removing the enamel cap. The number of these external bands matched the number of enamel bands. However, because of the relative ease in counting bands on the enamel cap, the use of external base bands (hereafter called “external bands”) has not been investigated.

Holden and Meadows (1962) suggested that spine growth resembled the stacking of paper cups. However, the growth of the *C. crepidater* spine is assumed to be similar to that of *S. acanthias* as described by Beamish and McFarlane (1985). In this case, upward growth is caused by deposition of dentine at the spine base, and outward spine growth is caused by the production of cartilage and dentine at the spine centre. Therefore the formation of external bands (and enamel bands) is independent of inner dentine band formation.

This study reports an alternative method of ageing deepwater dogfishes and constitutes the first investigation into the age and growth of any deepwater shark in the southern hemisphere. More traditional ageing methods using internal bands were also tried, and the results were compared with those of our technique.

**Materials and methods**

**Sample collection**

Between November 2000 and July 2002, *C. crepidater* were opportunistically collected from the bycatch of commercial fisheries operating in southeastern Australia (Fig. 1). Fishers were primarily targeting orange roughy (*Hoplostethus atlanticus*) and most *C. crepidater* were collected from depths of 650–1000 m.

Each dogfish was sexed, and the total length (TL) and fork length (FL) were measured (±1 cm) by allowing the caudal fin to take a natural position. All animal lengths hereafter are TL. For those dogfishes with a damaged caudal fin, FL was converted to TL using $TL = 1.08FL + 2.74$ ($n = 360$, $r^2 = 0.99$). Dogfishes were weighed (±10 g) on a top-loading digital scale, and the relationship between weight and length was examined. Embryos were weighted to the nearest 0.1 g and length was measured to the nearest 0.1 cm.

**Spine preparation**

Preliminary investigations found that the first dorsal-fin spines were more often damaged compared with the second dorsal-fin spines; therefore, second dorsal-fin spines were chosen for further examination. Second dorsal-fin spines were collected by cutting towards the vertebral column. Care was taken to include the delicate base portion. Spines were labelled and stored frozen for later examination.

Most of the muscle and connective tissue was removed from each spine using a sharp scalpel. Spine morphometrics (Fig. 2a) were measured using digital calipers (±0.01 mm). The external spine length (ESL) was measured from the tip of the spine to the point of entry into the flesh, and external spine width (ESW) was the diameter of the spine at this point of entry. Spines were thoroughly cleaned by repeatedly douching in hot tap water for a few seconds and then lightly scrapping with a blunt dental tool. Total spine length (TSL) was measured from the spine tip to the anterior side of the spine base. These morphometrics were used to examine spine...
growth by investigating their relationship with animal size. Spines were kept wet or frozen until all external age analysis had been completed.

External base bands were enhanced by soaking in a solution of saturated alizarin red in 1% potassium hydroxide (KOH) at a ratio of 1:100 (1% v/v) for 3–5 days. After the first 24 h, the internal cartilage rod could be removed easily. Spines were rinsed in tap water and were either examined immediately or stored frozen. Band clarity could be increased by lightly polishing with wet fine-grade abrasive paper. All spines were examined under a low-power (6×) dissecting microscope, with a magnifying lamp, by the naked eye, or a combination of all three depending on the particular spine.

One external band describes either a ridge of enamel or a white band after rubbing a stained spine with wet abrasive paper. Counting started at the spine base and some spines showed better band clarity along the posterior edge. Staining did not always enhance bands on spines from younger or faster-growing fish, as the bands were widely spaced and ridges were less obvious. In some older fish, bands near the base were very tightly packed and could only be identified on the posterior margin of the spine.

On completion of all external band examination, the base of the spine was “plugged” with plasticine and placed laterally in Wonderflex® silicon cookware “brownie” molds and embedded in epoxy resin (Renlam M-1 AU resin and HY951 hardener at 9:1 w/w). Plugging the spine base prevented resin from moving into the pulp cavity, which could cause damage to the internal spine structure. About 5–15 transverse sections were taken from the tip of each spine using a lapidary saw (rpm = 1250) fitted with a diamond-tipped 0.6 mm wide blade. Section thickness was measured (±50 µm) with digital callipers and was generally 250–350 µm. The internal spine structure consists of three dentine layers: outer, middle, and inner (Fig. 2b). However, the internal dentine layer was much wider than the two other layers and had the clearest bands. The optimal sectioning point was immediately below the apex of the pulp cavity. Spine sections were mounted onto glass slides using epoxy resin. Slides were placed in a 35 °C oven until the resin had set (usually taking 1–2 h).

Bands are formed simultaneously in each of the three dentine layers (McFarlane and Beamish 1987). Band spacing is widest in the inner layer, and therefore, only bands in the inner dentine layer were counted. Counting started at the pulp cavity (spine centre) and continued outwards until the first trunk primordium (the junction between the inner and middle layers). One internal growth band refers to a dark (opaque) and light (translucent) concentric band.

Reading precision and accuracy

Three nonconsecutive band counts were made for each spine without prior knowledge of the animal’s length or sex or the previous band counts. A subjective measure of band readability was used: the sliding scale started at 1 (samples with unambiguous bands with excellent readability) to 5 (unreadable sample). An independent reader was not available, and therefore, approximately 20% of the samples were re-examined 1–3 months after the first examination to imitate the second (independent) reader protocol suggested by Cailliet and Goldman (2004).

Precision was calculated using the coefficient of variance (CV) across all fish ages following Campana (2001). Calculating CV gave an assessment of the ease of ageing dogfish spines and tested the within-reader reproducibility of age determinations. Samples were discarded if the readability score was >3, and an upper limit for CV was set at 20% for each spine section (adapted from the index of average percent error (IAPE) for vertebrae analysis; Beamish and Fournier 1981). Samples were not included in the analysis if the CV was >20%. The average of the mean age for each of the three counts defined the age estimate for each shark (Casey et al. 1985).

Verification

The annual periodicity of internal bands was investigated by edge analysis and marginal increment analysis. An edge-grading system adapted from Yudin and Cailliet (1990) was applied for edge analysis. The condition of the growing edge of the inner dentine was examined under 60× to 100× magnification and was related to the season of capture. Edges

Fig. 2. (a) Dorsal fin spine illustrating morphometric measurements: total spine length (TSL), external spine length (ESL), and external spine width (ESW). (b) Internal structure of a dorsal-fin spine illustrating the three (inner, middle, and outer) dentine layers.
were recorded as translucent, narrow opaque, or wide opaque. The observed and expected ratios of translucent to opaque last bands for each season were then compared using chi-square ($\chi^2$) tests.

The marginal increment ratio (MIR) was calculated from transverse spine sections with 16–19 internal bands, where the width of the ultimate band ($w_b$) was expressed as a proportion of the penultimate band ($w_{b-1}$). All measurements (µm) were made using the F-View Soft Imaging System (SIS, 8 Timbertop Crt., Gulfview Heights, SA 5109, Australia; www.soft-imaging.net) on a BX51 Olympus compound microscope with a differential interference contrast. The radius of the inner dentine section was used to ensure that sections from the same regions were examined. Measuring the marginal increment in the inner dentine was difficult. Extremely thin (<300 µm) sections were required to ensure that the depth of field was minimised at high magnification. Mean MIR ± 1 standard error (SE) was plotted seasonally to locate periodic trends in band formation. If the translucent zones are formed annually, the MIR should decline once each year (i.e., when the new opaque zone starts to form outside the translucent zone). Analysis of variance (ANOVA) was performed to detect any significant differences in the MIR throughout the year.

The length-at-age data and growth curves were compared with published ages from radiometric isotope analysis of vertebrae for five female C. crepidater from southern Australia (Fenton 2001).

**Growth**

The von Bertalanffy growth model (VBGM) is commonly used to represent fish growth, although it does not always provide a particularly good fit and there has been a wide array of criticisms (Haddon 2001). Moulton et al. (1992) adopted the Francis (1988) reparameterized VBGM equation (eq. 1) for shark age and growth analysis to correct for the effects of gillnet length-selective sampling bias. The reparameterized equation was subsequently used to test for the biasing effects of gillnet length-selective fishing mortality (Walker et al. 1998):

$$L = L_\infty + (L_w - L_\infty)(1 - e^{-k(T - t_0)})(1 - r^2)$$

where the three von Bertalanffy (1938) parameters ($L_\infty$, $t_0$, and $k$) are replaced with $L_w$ (mean length at reference age $\phi$), $L_\infty$ (mean length at reference age $\psi$), $r$ (mean length at reference age ($\phi + \psi$)/2), and $r = (L_w - L_\infty)(L_\infty - L_\psi$. The same reference ages ($\phi$ and $\psi$) were chosen for males and females within the range of the data to avoid unnecessary extrapolations and to allow direct comparisons between male and female growth.

The Francis model was used to examine growth for each sex, and the Francis parameters were related to the conventional von Bertalanffy parameters using

$$L_\infty = L_w + (L_w - L_\phi)/(1 - r^2)$$

$$k = -(2 \log_e r) / (\psi - \phi)$$

$$t_0 = \phi + (1/k) \log_e (L_\infty - L_\phi)/L_\infty$$

Confidence intervals (95%) around the Francis parameters gave a direct measure of the heterogeneity in length at age. Confidence intervals for both Francis and VBGM parameters were calculated using the nonlinear regression function in Systat 8.0® (Systat Software Inc. 1999).

Francis growth parameters were directly compared, although $\chi^2$ tests on each likelihood ratio were used to compare the data between sex and external versus internal band counts. This method, advocated by Kimura (1980), Moulton et al. (1992), and Haddon (2001), is a more reliable means of finding a difference in growth. Chi-squared tests were performed in Microsoft Excel® as outlined by Haddon (2001).

The relationship between external and internal band counts was investigated by regression analysis using the nonlinear (mode/loss) regression function in Systat 8.0® (Systat Software Inc. 1999).

**Longevity**

Longevity was assumed to be the maximum number of external band counts.

**Age at maturity**

Sexual maturity in males was determined by clasper condition (elongation and calcification) and macro-examination of the testes. Males were considered mature once claspers had fully calcified and testes showed signs of lobulation. The reproductive status of females was based on the condition of the ovaries and uteri adapted from Wetherbee (1996) and Stehmann (2002). Females were assumed in a mature condition when distinct oocytes were present in the ovaries and (or) the uteri had expanded away from the central axis of the body cavity.

The age at maturity ($A_{50}$) was estimated from the relationship between the proportions of mature versus immature specimens within 5-year age classes. Each interval was indicated by its lower value. A logistic curve was fitted for each sex using probit analyses.

**Results**

The relationship between weight ($W$) and total length (TL) differed significantly with sex ($p < 0.05$): male, $W = 0.001 \text{TL}^{3.454}$ ($r^2 = 0.67, n = 154$); female, $W = 0.002 \text{TL}^{3.234}$ ($r^2 = 0.86, n = 238$). Length ranges were 30–79 cm for...
males and 29–99.5 cm for females. The length frequencies indicate sexual dimorphism, with females attaining a larger size.

Spine structure and growth

The first and second dorsal-fin spines of C. crepidater are similar in size and structure. Each spine is slightly curved and the portion beneath the skin is ivory in colour, whereas the external portion is usually dark brown. A small strip of enamel covers the anterior dentine portion, and no other enamel is present on the spine surface. The cartilage rod that supports the spine is about two-thirds of the total spine length.

Spines were visible in embryos >10 cm TL, and full-term embryos (~30 cm TL) had fully developed spines measuring 8–9 mm TSL. A total of 344 second dorsal-fin spines were collected and measured, of which 267 had no damage, 55 had a worn or blunt tip, 18 had a broken tip, and four were broken and therefore discarded. The relationship between TL and TSL for nondamaged spines was linear and differed significantly between sexes ($p < 0.05, F = 9.98$): male, TL = 1.60 TSL + 16.02 ($r^2 = 0.67, n = 113$); female, TL = 1.12 TSL + 29.44 ($r^2 = 0.59, n = 142$).

Approximately 75% of the spine was beneath the skin. Spine growth was most rapid between 30 cm (birth) and 40 cm TL (Fig. 3), and the linear correlation strengthened significantly ($r^2 = 0.99$) after 40 cm TL for both sexes.

External bands

The external surfaces of 257 second dorsal-fin spines were examined for growth bands. A countable banding pattern (Fig. 4) was found on the surface of 241 (94%) spines. Overstaining was the main cause of poor readability, and most spines had a readability score of 1 or 2. The average reading precision (CV) of readable counts was 4.04%. Ten ages had a CV above 20% and were excluded from all age analyses. The number of bands did not influence readability, and there was very poor correlation ($r^2 = 0.054, n = 229$) between the number of bands and the coefficient of variance.

The number of external bands increased with animal size, and no bands were found close to the spine tip. The greatest number of bands observed was 54 (99 cm TL) for females and 34 (70 cm TL) for males. The largest female (99.5 cm TL) had 37 bands, and the largest male (79 cm TL) had 26 bands. Near-term embryos had no external bands, and a birth-band subtraction to estimate age was not required. The

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**Fig. 4.** Second dorsal-fin spine of Centroselachus crepidater stained with alizarin red and potassium hydroxide to enhance the external banding pattern (each asterisk (*) represents one band; scale bar = 10 mm).

**Fig. 5.** Reparameterized von Bertalanffy growth (VBG) curves generated from (a) external spine data for male (M, solid circles, $n = 86$) and female (F, open circles, $n = 146$) and (b) internal spine data for male (M, solid circles, $n = 69$) and female (F, open circles, $n = 100$) Centroselachus crepidater from southeastern Australia. Individual VBG and Francis parameters are given in Table 1.
base of larger (older) spines was fragile and was often only partly formed.

Assuming external bands are formed annually, the re-parameterized VBGM was fitted to length-at-age data for each sex (Fig. 5a; parameters are listed in Table 1). The Francis parameters show that males grow about 32 cm from the ages of 2 to 12, whereas females grow 40 cm over the same period. However, males only grew a further 4 cm over the next 10 years (between 12 and 22 years) compared with 14 cm for females.

Kimura’s likelihood ratio test calculated a difference in the growth curves for male and female *Centroselachus crepidater*. A strong difference between the $L_{\infty}$ of each sex was indicated ($p = 0.009$), whereas there was no indication that the $t_0$ and $k$ parameters differed significantly ($p = 0.569$ and 0.122, respectively).

The number of external bands ranged from 0 to 54 years for females and from 0 to 33 years for males. However, most females were 24–34 years, whereas males 16–24 years were more common.

**Internal bands**

Cross sections of 201 spines were examined, and the inner dentine layer contained the most distinguishable banding pattern (Fig. 6). The optimal sectioning location (where band clarity was the best) was where the inner dentine was the widest; this corresponded to a region ~5 mm from the tip in unworn adult spines. Sections close to the tip had poor band clarity and the middle to outer layers were very narrow.

The number of internal bands increased with animal size, and the greatest number of bands observed was 27 (83 cm TL) for females and 22 (72 cm TL) for males. The spines of the largest female (99.5 cm TL) and male (79 cm TL) examined had unreadable internal bands. The largest female with readable internal bands (93 cm TL) had 21 bands, whereas the largest male with readable bands (77 cm TL) had 14 bands.

The mean CV of these bands was 7.7%, and 32 spines (15%) were excluded because of poor readability or poor

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male (n = 86)</th>
<th>Female (n = 146)</th>
<th>Male (n = 69)</th>
<th>Female (n = 100)</th>
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<tr>
<td><strong>von Bertalanffy</strong></td>
<td></td>
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<tr>
<td>$L_{\infty}$ (cm)</td>
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<td>70.6</td>
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<td>100.9</td>
<td>71.8</td>
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<tr>
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<td>0.163</td>
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<td><strong>Francis</strong></td>
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<td>$l_{22}$ (cm)</td>
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**Note:** $L_{\infty}$, asymptotic length; $k$, growth coefficient; $t_0$, age at length 0; $r^2$, coefficient of determination; $l_2$, mean total length (TL) at age 2; $l_{12}$, mean TL at age 12; $l_{22}$, mean TL at age 22.
Correlation between external and internal growth bands

A strong curvilinear relationship ($r^2 = 0.936$) was found between the number of external and internal bands (Fig. 7), with the number of external bands exceeding internal bands in most samples. The polynomial relationship showed that the first five bands were deposited at the same rate. However, over the next 10 years (external band ages 5 to 15), only one internal band was deposited for every 1.25 external bands. After 15 years, this rate rapidly declined, and after the age of 30, no new internal bands were deposited.

The growth curves from external bands were significantly different to the growth curves from internal bands. Francis parameters greatly differed for males, and Kimura’s likelihood ratio test calculated a difference in the VBGM $k$ parameter for each sex (male $p = 0.023$; female $p = 0.015$), whereas $L_{\infty}$ also differed between the curves for males ($p = 0.007$).

Verification

The inner dentine layer of samples collected in spring had more wide opaque marginal edges than other edges (Fig. 8a), although a trend of annual band deposition could not be confirmed ($\chi^2 = 1.32$ (NS)). A high mean MI ratio was calculated for each season (Fig. 8b), and MI did not differ significantly between seasons (ANOVA, df = 3, $F = 0.744$, $p = 0.538$). The small number of readable spine sections with 16–19 bands ($n = 30$) limited the use of marginal increment analysis, and both edge and marginal increment analyses failed to confirm a distinct seasonal pattern in band deposition.

The radiometric ages presented in Fenton (2001) were similar to the external band age estimates from this study (Fig. 9). There was no significant difference between the length-at-age data from external bands and radiometric age data from vertebrae (ANOVA, $p = 0.31$, $F = 1.07$).

Longevity

Based on the maximum number of external bands ($A_{\text{max}}$), female *C. crepidater* live about 20 years longer than males; the oldest male examined was 34 years old and oldest female was 54 years old.

Age at maturity

External band counts indicate that females mature ($A_{50}$) at about 20 years (SE = 0.5) (Fig. 10); this is 37% of $A_{\text{max}}$. Maturity occurred over a very broad age range (12–42 years), although the youngest female in the early stages of pregnancy (fertilised eggs in utero) was 27 years old, whereas the youngest pregnant female with near-term embryos was 29 years old.

Females matured over a wider age range than males. External band counts indicate that male maturity occurred over an 8-year period, the youngest mature male was 9 years old, and the oldest immature male was 17 years old. Male $A_{50}$ was estimated to occur at the age of ~9 years (SE = 1.4) (Fig. 10), which is 26% of $A_{\text{max}}$.

Discussion

Dorsal spines affect the hydrodynamics of the dorsal fin and offer a degree of protection from predation (Maisey 1979). *Centroselachus crepidater* embryos have fully formed spines, although most of the spine is beneath the skin. After birth, external spine growth is rapid. A smaller external spine in utero may reduce the risk of injury to both mother and siblings, whereas a larger spine after birth may have a defensive and locomotory function.

Growth increments

The relationship between animal length and spine length suggests that *C. crepidater* spines continue to grow throughout life, making them a suitable structure for estimating age and investigating growth. Internal bands, similar to those reported by Tanaka (1990), were found in spine cross sections. However, external bands proved to be an alternative (and probably more accurate) method to estimate age.

Fig. 7. Relationship between external bands (EB) and internal bands (IB). Plots are means, and error bars represent ±1 standard error. The polynomial relationship was IB = −0.011(EB$^2$) + 0.813(EB) + 1.552 ($r^2 = 0.918$, $n = 111$). Broken line indicates the exponential relationship if 1 EB = 1 IB.
Holden and Meadows (1962) overlooked the external bands on *S. acanthias* spines because of the ease of counting enamel bands. Maisey (1979) examined the structure and function of selachian fin spines and reported faint growth lines at the spine base of squalid (*Squalus, Etmopterus,* and *Deania* species) and heterodontid spines. External bands have also been noted on the spines of *Centroscymnus owstoni*, *Centrophorus uyato*, *Centrophorus squamosus*, *Deania calcea*, *Deania quadrispinosa*, *Etmopterus baxteri*, *Etmopterus* sp. B (Last and Stevens 1994), *Etmopterus lucifer*, *Etmopterus pusillus*, *Oxynotus bruniensis*, *Proscymnodon plunketi*, *Squalus megalops*, and *Heterodontus portusjacksoni* from southeastern Australia (S. Irvine, personal observation). These observations suggest that external bands on the spine may be suitable for ageing numerous shark species, and their usefulness in estimating age should be examined in future age and growth studies. Staining may not be necessary or beneficial for some spines (e.g., alizarin red only superficially stains *Deania calcea* spines and this stain decreased the band readability; S. Irvine, personal observation).

Both internal and external bands increased in number with animal length, although most spines had more external bands. The relationship between internal and external counts suggests that bands only formed at the same rate in the first 5 years. Internal band formation dramatically declined after 20 external bands had formed, and internal bands cease to form after 30 years (based on external bands). Maisey (1979) could not find a venous return system in adult spines and suggested that the inside of adult spines stop growing when the space above the pulp cavity has been filled by dentine. As spine growth rate slowed, the inner bands became more narrowly spaced in larger and (or) older animals. External bands also become more tightly packed, and larger and (or) older animals had spine bases with very little...
dentine. However, the number of external bands did not affect spine readability or reading precision.

Soldat (1982) and Nammack et al. (1985) used different growth areas of the spine to investigate the age of *S. acanthias* from the Northwest Atlantic. Soldat counted the internal bands in spine cross sections and obtained maximum ages of 20 (males) and 26 (females) years, while Nammack et al. used the enamel cap bands and reported maximum ages of 35 (males) and 40 (females) years. Nammack et al. suggested that the difference might be due to internal bands being grouped together as annuli during counting by Soldat. However, the growth curves predicted by these studies are remarkably similar, and internal and external bands were counted at a similar rate until 20 internal bands; internal bands then ceased to form at the same rate as external bands. Beamish and McFarlane (1987) validated the periodicity of enamel cap bands on *S. acanthias* from the Northeast Pacific through mark and recapture with tetracycline injection. A tetracycline mark was observed on the enamel, at the spine base, and within the inner dentine layer, and it was assumed that internal dentine bands were also annual. However, this assumption requires verification across all age and size ranges, as internal bands may not form annually in spines of older *S. acanthias*.

**Precision and verification**

The precision of the age data as indicated by the CV values showed that external bands were easier to interpret than internal bands. External bands form because of the lack of synchrony in upward spine growth and the continuous production of dentine at the spine base. Investigating the periodicity of external bands was not attempted (by either edge or marginal increment analyses), as external bands are not deposited in the typical opaque or translucent band pattern. However, enamel and internal dentine bands may be suitable for either edge or marginal increment analysis.

Marginal increment analysis was attempted on spines with 16–19 internal bands. Guallart Furio (1998) suggested that increment analysis would be impossible on squalid spines, as obtaining sections from the same spine location is required. To ensure spine sections from a similar location were used for increment analysis, the diameter of the spine section and the inner layer radius were measured. Marginal increment analysis was attempted on the internal bands, although the periodicity of band formation was inconclusive because of the small number of animals collected in the appropriate age class each month or season. The high longevity of this species and the wide age range suggests that a very large (*n* > 1400) sample size would be required to accurately attempt either edge or marginal increment analysis on a single age class of *C. crepidater*. Verifying band periodicity in spines using marginal increment analysis or edge analysis is likely to remain problematic, especially when the study species have a high longevity or when samples are collected opportunistically over a limited sampling period.

The applicability of independent ageing techniques (including radiometric analysis and radiobomb carbon dating) on the dorsal-fin spine also deserves further attention. External spine age estimates for female *C. crepidater* were comparable with the absolute ages of five large females estimated by radiometric analysis from vertebrae (Fenton 2001). The results of Fenton (2001) are based on many assumptions, and Welden et al. (1987) suggested that elasmobranch vertebrae were unsuitable for radiometric analysis. However, the similarity between the two data sets indicated that further research into radiometric ageing of shark vertebrae is required.

McFarlane and Beamish (1987) reported the incorporation of tetracycline into *S. acanthias* spines, making them a suitable structure to age after chemical tagging. However, very little is known about the survivability rate or chance of recapture of deepwater sharks. Yano and Tanaka (1986) successfully tagged and tracked two deepwater dogfishes (*Centrophorus acus*) caught by drifeline, although this tracking study was abandoned after 24 h. Deepwater sharks may therefore survive being brought to surface waters to allow conventional tagging and tetracycline injection for ageing purposes.

**Growth**

The cue for growth (and therefore band deposition) in the deep sea is unknown. An annual cycle in food quantity and quality is often assumed (Clarke 2000; Swan and Gordon 2001), although seasonal dietary changes have not been investigated for deepwater dogfishes. Off southern Tasmania, there is no seasonal change in the species composition for the more abundant species, although micronekton composition does vary seasonally (Williams and Koslow 1997). This suggests that, if a change in diet is the cue for growth, it may be an increase in prey abundance (quantity) rather than a change in prey species (quality).

The Francis (1988) reparameterized von Bertalanffy growth model fits the observed length-at-age data well. Male and female growth was similar until males became mature. Males matured (*A*<sub>50</sub>) at about 9 years, whereas females matured at about 20 years. However, maturity analysis required samples to be put into age classes represented by their lower values; age at maturity may therefore best be indicated over an age range.

**Maturity**

The estimated age at maturity differed from the age at maternity (the youngest pregnant female). Females matured at about 20 years, although the age of youngest female with candled uteri (early pregnancy) was 27 years and the age of the youngest female with near-term embryos was 29 years. Walker (2004) suggested the use of a maternity ogive rather than a maturity ogive for estimating recruitment in population dynamic models.

However, this becomes difficult for species that have an unknown gestation period and resting stage between pregnancies. There is no difference in the size at maturity (*L*<sub>50</sub>) and the size of the smallest pregnant female (both 82 cm TL) (S. Irvine, unpublished data), suggesting that females might not grow during reproductive activity. Further growth may only occur during the resting period (between pregnancy and next follicle development phase).

**Productivity**

*Centroscelus crepidater* from southeastern Australia exhibits slower growth (Fig. 11) and higher longevity than most other dogfish species. However, *C. squamosus* from the Northeast Atlantic has a longevity of 70 years (Clarke et al.
Fig. 11. Various von Bertalanffy growth curves for female dogfish species: a, Centroscyllium atra, this study; b, Squalus acanthias, Nammack et al. (1985); c, Centrophorus acus, Tanaka (1990); d, Centrophorus granulosus, Guallart Furio (1998); e, Deania calcea, Clarke et al. (2002b).

2002a) and remains the oldest dogfish species ever aged (albeit unvalidated).

Centroscyllium atra from southeastern Australia has a mean litter size of six and the reproductive cycle is non-continuous with no seasonal trend (Daley et al. 2002). Although the length of gestation is unknown, it seems reasonable to assume that the reproductive cycle (follicle development and gestation and resting period) would exceed 1 year and is probably 2 or more years. If a cycle of 2 years were assumed, an annual fecundity of three would indicate a maximum productivity of 102 offspring per lifetime. However, if the cycle were 3 years, then the maximum productivity would only be 68 offspring per lifetime.

The resilience of a species to fishing pressure depends on vulnerability to the fishing gear and biological productivity (Stevens et al. 2000). The high longevity and late age at maturity of C. crepidater from southeastern Australia are indicators of low productivity. Smith et al. (1998) assessed the productivity of 26 shark species using published age at maturity and maximum reproductive age data. According to this rank of sensitivity to fishing pressure, C. crepidater has a lower recovery capability than all of the Pacific shark species examined by Smith et al.

Nonselective trawl fishing off southeastern Australia collected a wide age range of C. crepidater, although most females had only recently matured (24–34 years old). The removal of this crucial component is likely to be unsustainable, and precautionary management regimes (including closed areas) need to be implemented immediately to avoid local extirpations. In light of this new information on age and growth, international management and conservation attention need to be directed towards dogfishes.

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