

Comparison of Whole and Sectioned Vertebrae for Determining the Age of Young Blue Shark (*Prionace glauca*)

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Abstract

Age determinations using whole and sectioned vertebrae from 185 blue shark (*Prionace glauca*) showed no systematic between-method difference in ages to a minimum age of 8 years. The precision measure, coefficient of variation, was good for both methods (CV <8%). While age validation is still required, digitally-enhanced images of whole vertebrae appear to be a rapid and cost effective means of age determination for blue shark below 300 cm (fork length) or approximately age 8.

Keywords: age-bias, age determination, precision, *Prionace glauca* vertebrae

Introduction

The blue shark (*Prionace glauca*) is among the widest ranging of all shark species, being found in every temperate and tropical ocean (Compagno, 1984). The species is thought to grow quickly, maturing at approximately 185 cm (*fork length*: FL) or about age 5 (Pratt, 1979). However, estimated growth rates have varied among studies, possibly because of the range of ageing methods which have been applied (Aasen, 1966; Cailliet *et al.*, 1983; Stevens, 1975; Tanaka *et al.*, 1990). Although the accuracy of the age interpretations has been validated in several shark species, there is as yet no validated method of age determination for blue sharks (Natanson and Cailliet, 1990; Brown and Gruber, 1988).

Most length-at-age information for shark species worldwide has come from the interpretation of growth bands visible in vertebral structures (Cailliet, 1987). Past authors have examined whole and/or sectioned vertebrae in their studies (Tanaka *et al.*, 1990; Cailliet *et al.*, 1983; Stevens, 1975). These differences in method may contribute to the apparent differences in

growth rate observed among blue shark ageing studies.

The objective of this study was to compare age determinations of blue shark based on whole and sectioned vertebrae using image analysis methods. For simplicity we will use the term *ages* to denote our *age estimates* as the ageing of blue shark vertebrae has not yet been validated.

Materials and Methods

Samples of blue shark vertebrae were obtained from 185 fish caught during sport-fishing tournaments in Nova Scotian waters off the east coast of Canada in August–September 2000. At dockside, sharks were measured (FL, measured over the body curve) to the nearest cm, maturity assessed and vertebral samples were taken from each fish within hours of being caught. Cervical vertebrae ($n=4$) adjacent to the most posterior of the gill slits, were removed from each shark upon landing, then frozen for transportation and storage at -27°C . In the laboratory, vertebrae were cleaned of adhering soft tissue using a scalpel. Digital

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images of each whole vertebral face were obtained under reflected light using a Kodak Megaplus 1.4 digital camera at a resolution of 1280×1024 pixels and Optimas 6.1 video capturing software. After whole images had been collected, the vertebrae were sectioned along their midsagittal axis to a thickness of 0.7mm with an Isomet slow speed saw and digitally photographed under reflected light. Vertebral images were subsequently enhanced using Adobe Photoshop 5.0 and aged in a blind/independent manner by two readers. Whole vertebrae were aged independently of sectioned vertebrae.

Age-bias plots were generated for analysis of whole/sectioned, inter-ager, and intra-ager results. Age-bias plots provide a clear, simple means of detecting bias and are excellent for illustrating matched pair relationships. Matched pairs are compared by plotting the mean counts of one ager for the samples corresponding to each of the age categories of the other ager. A 1:1 line of equivalence is included to indicate where $\text{ager } x = \text{ager } y$. The extent of deviation of the 95% confidence interval bars from the 1:1 equivalence line indicates the extent of ageing bias (see Campana *et al.*, 1995).

Precision estimates for each method of each ager were calculated using the coefficient of variation (CV) as per Chang (1982). Once all vertebral ageing was complete, 30 whole and 30 section images were randomly taken and re-aged by both agers to obtain replicate values for the calculation of CV. The CV was calculated as:

$$CV_j = 100 \times \frac{\sqrt{\sum_{i=1}^R (X_{ij} - X_j)^2 / (R-1)}}{X_j}$$

where X_{ij} was the i th age determination of the j th fish, X_j was the mean age of the j th fish and R was the number of times each fish was aged. This equation was then averaged among all fish to give a mean CV for both the whole and sectioned methods per ager.

Growth estimates were calculated based on the von Bertalanffy growth model (von Bertalanffy, 1938; Beverton and Holt, 1957):

$$L_t = L_\infty * [1 - \exp(-K * (t - t_0))]$$

where L_t is length at age t , L_∞ is the asymptotic length, K is the growth rate parameter and t_0 is a theoretical age at which the length is equal to zero. All lengths for comparison with other published von Bertalanffy

estimates were first converted to straight total length (TL) using the conversion formula: $TL = (FL - 1.73872)/0.82995$ (Pratt, 1979).

Results

The lengths of the male blue sharks examined ranged between 147–282 cm, but were bimodally distributed with peaks at 170 and 240 cm (Fig. 1). Female blue sharks were smaller, ranging between 145–195 cm and a mode at 170 cm.

Both the whole and sectioned vertebrae (Fig. 2) showed banding patterns of decreasing width from the centre to the edge of each vertebra. The high magnification of the section images allowed for easy resolution of the thinnest bands near the vertebral edge whereas these bands were somewhat compressed in the whole images. Conversely, bands in the whole images were better defined than the diffuse pattern observed in the section images.

The age-bias plot comparing sectioned versus whole ages (Fig. 3) showed no consistent bias between the two ageing methods across the age range of 2–8 years. Therefore, whole and sectioned vertebrae provided comparable age estimates, at least on average. However, the widths of the confidence intervals for ages 2, 7 and 8 were relatively broad, suggesting that the level of agreement for the youngest and oldest ages was considerably less precise than that for the intermediate ages. The precision estimates (CV) were 5.9% for the section method and 7.7% for the whole method suggesting they are both highly reproducible.

The von Bertalanffy growth parameters based on whole and section ages (males and females pooled) were similar in magnitude to those reported in other studies (Aasen, 1966; Stevens, 1975; Cailliet *et al.*, 1983; Tanaka *et al.*, 1990; Henderson *et al.*, 2001). Calculated von Bertalanffy growth parameters were $L_\infty = 300$; $K = 0.68$ and $t_0 = -0.25$ for whole ages and $L_\infty = 302$; $K = 0.58$ and $t_0 = -0.24$ for section ages. The resulting von Bertalanffy growth curves were similar for whole and section ages (Fig. 4).

Discussion

The comparison between whole and sectioned vertebrae indicated that the two methods produced comparable ages until at least age 8, which corresponded to lengths beyond the reported size of sexual maturity (Pratt, 1979). However, inspection of whole vertebrae suggested that inadequate resolution of the narrow

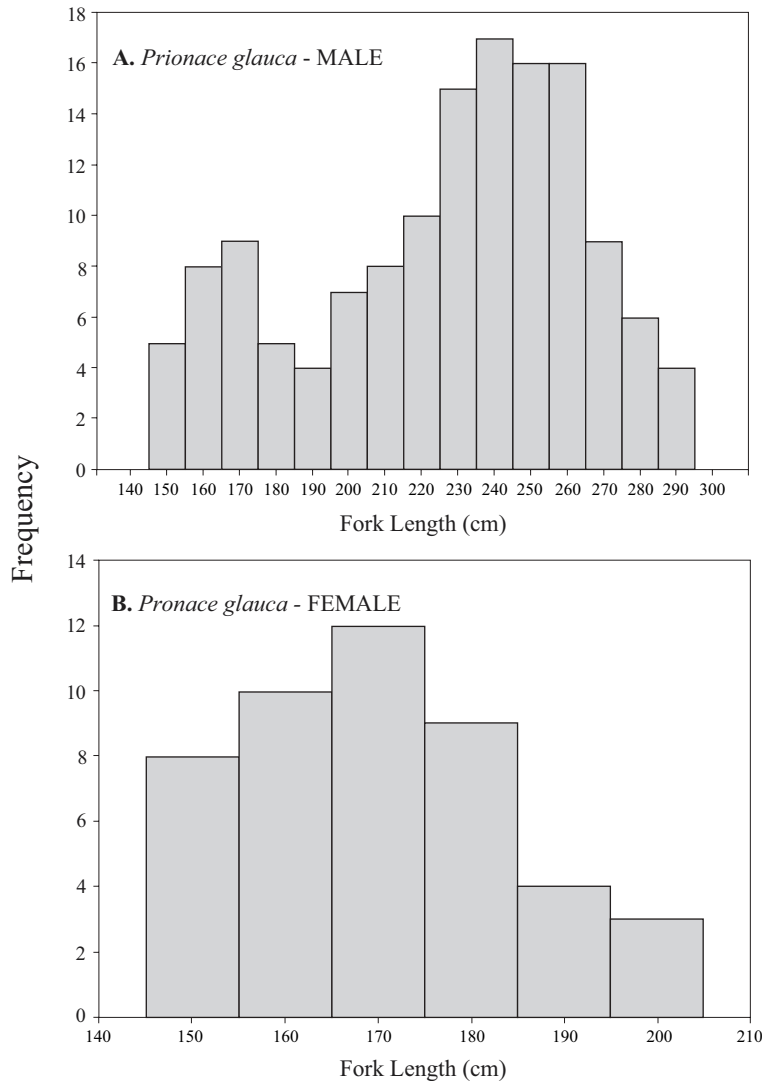


Fig. 1. Length-frequency distribution of sampled (A) male and (B) female blue shark from Nova Scotian waters.

outer bands could be a possible source of ageing bias in older sharks. Blue shark growth declines in older individuals, as is typical for most fishes. Because of the curved surface of the vertebral face, the decreasing width of the most recently-formed growth bands were more difficult to distinguish, resulting in the possible aggregation of adjacent bands during ageing. Age comparisons within and between age readers showed that this had indeed occurred with the less experienced ager in the study, whereby the whole counts of the one ager were shown to be under-aged relative to both the section counts and the counts of the other ager. Presumably this type of error would be less likely with experienced agers. Nevertheless, given these results, we cannot discount the possibility that

the whole vertebrae might under-estimate ages in blue sharks older than age 9.

The length-frequency distributions of both males and females were characterized by modes around 170 cm, corresponding to immature sharks. In the case of the females, this was the only mode present. However the length distribution for males was bimodal, with the larger mode corresponding to mature animals. Thus mature females appear to be absent from Nova Scotian waters during the late summer months.

The ageing precision based on sections (5.9%) was slightly better than that based on whole vertebrae (7.7%), although both whole and sectioned vertebrae

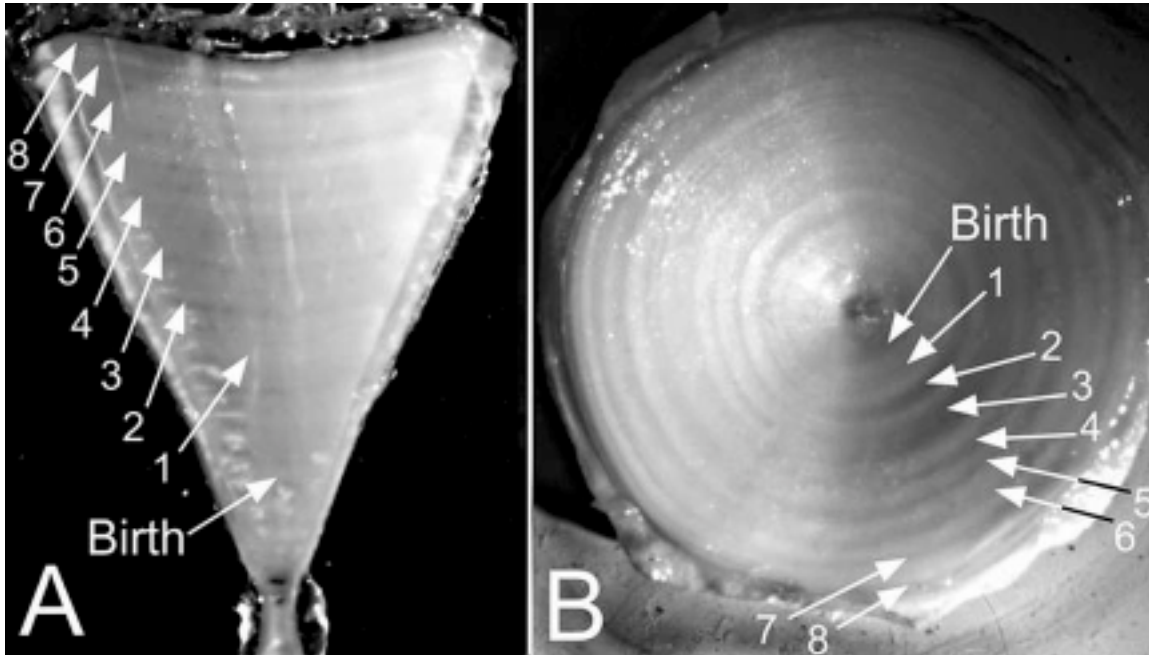


Fig. 2. Images of a single *Prionace glauca* vertebra under reflected light in both (A) sectioned and (B) whole form. Age determinations are annotated and the birth band noted.

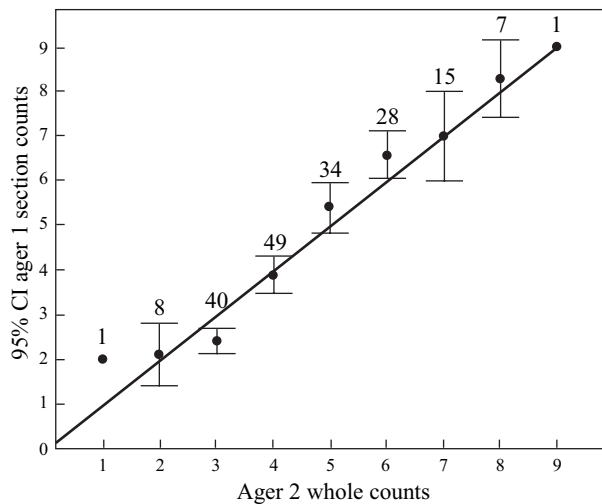


Fig. 3. Age-bias plot comparing ages from whole and sectioned blue sharks vertebrae. The extent of deviation of the 95% confidence interval bars from the 1:1 line indicates the extent of ageing bias.

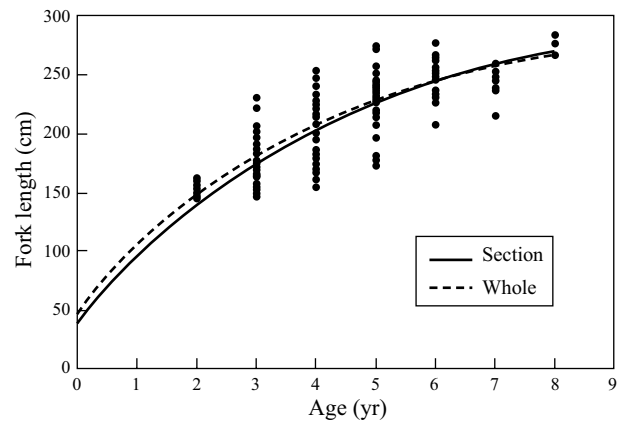


Fig. 4. Von Bertalanffy growth curves for 185 blue sharks (sexes combined) based on whole and sectioned vertebrae. The calculated von Bertalanffy growth equations are: $L_t = 300(1 - \exp[-0.68(t+0.25)])$ for whole ages and $L_t = 302(1 - \exp[-0.58(t+0.24)])$ for section ages.

resulted in more precise ages than the 9–10% mean CV value of past vertebra-based studies (Campana, 2001). Therefore both methods were highly reproducible, and when combined with the age-bias results, suggest that they provide analogous ageing rigor.

These results were consistent with those of Tanaka *et al.* (1990), who found no consistent differences between stained whole vertebrae and vertebral sections, although they did find differences among laboratories.

In calculating von Bertalanffy growth parameters for our study, we chose to group the male and female data together, based on the absence of mature females. We felt this to be appropriate as studies which have separated males and females have produced inconsistent differences in parameter estimates between sexes, e.g. some show higher L_{∞} values for males (Cailliet *et al.*, 1983; Tanaka *et al.*, 1990) and others for females (Skomal, MS 1989). The grouped parameters calculated in this study did nonetheless, appear to be similar in range to other studies (Aasen, 1966; Skomal, 1989; Stevens, 1975; Cailliet *et al.*, 1983; Tanaka *et al.*, 1990; Henderson *et al.*, 2001). Plotted curves gave comparable results for both whole and section ages (Fig. 4), an unsurprising result given the close agreement noted by the age bias plots. These curves and estimates are provisional however, as there were few older individuals in our study and no validation has been done. They served principally to ensure our estimates did not differ wildly from previous work.

Tanaka *et al.* (1990) demonstrated that intra- and inter-reader error, as well as differences among methods, could account for most differences in age interpretation. As outlined above, our intra- and inter-reader precision was minimal, thereby removing these as substantive sources of variation. Tanaka *et al.* (1990) also acknowledged inter-oceanic growth differences as a possible source of age estimation differences. Seasonality of temperature and growth in temperate areas are thought to cause the strong banding patterns observed in many temperate species (Stevens, 1975). However, since all previous studies have been carried out in temperate waters (Cailliet *et al.*, 1983; Stevens, 1975; Aasen, 1966; Henderson *et al.*, 2001; Tanaka *et al.*, 1990) it is not known if distinct banding is also present in tropical blue shark. In the absence of age validation studies, our agreement with other studies may be due more to similarities in method than proximity to true ages; i.e. precision does not denote accuracy (Campana, 2001). Until validation work is completed on blue shark age estimates, the accuracy of all reported results will continue to be unknown.

The high resolution digital images produced with the image analysis system provided excellent detail and clarity for ageing purposes and avoided the need for dyes or stains (e.g. Cailliet *et al.*, 1983). Use of whole vertebrae proved to be the easier of the two ageing methods; the resulting images showed clear bands which were acquired in a short period of time

using less specialised equipment than those required for the sections. Given the similar ages produced by the two ageing methods, any age validation study which confirms the accuracy of one method must necessarily confirm the accuracy of both methods. Therefore, it is age validation which is now required for age determination of young blue shark.

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