# Reassessment of spiny dogfish *Squalus acanthias* age and growth using vertebrae and dorsal-fin spines

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Male and female spiny dogfish *Squalus acanthias* were collected in the western North Atlantic Ocean in the Gulf of Maine between July 2006 and June 2009. *Squalus acanthias* ranged from 25 to 102 cm stretch total length and were caught during all months of the year except January. Age estimates derived from banding patterns visible in both the vertebrae and second dorsal-fin spines were compared. Vertebral growth increments were visualized using a modified histological staining technique, which was verified as appropriate for obtaining age estimates. Marginal increment analysis of vertebrae verified the increment periodicity, suggesting annual band deposition. Based on increased precision and accuracy of age estimates, as well as more biologically realistic parameters generated in growth models, the current study found that vertebrae provided a more reliable and accurate means of estimating age in *S. acanthias* than the second dorsal-fin spine. Age estimates obtained from vertebrae ranged from <1 year-old to 17 years for male and 24 years for female *S. acanthias*. The two-parameter von Bertalanffy growth model fit to vertebrae-derived age estimates produced parameters of $L_\infty = 94.23$ cm and $k = 0.11$ for males and $L_\infty = 100.76$ cm and $k = 0.12$ for females. While these growth parameters differed from those previously reported for *S. acanthias* in the western North Atlantic Ocean, the causes of such differences were beyond the scope of the current study and remain to be determined. © 2011 The Authors

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## INTRODUCTION

The spiny dogfish *Squalus acanthias* L. 1758 is a shark species found in the Atlantic and southern Pacific Oceans (Burgess, 2002). Recently, female *S. acanthias* were heavily targeted in a fishery on the east coast of the U.S.A. (NEFSC, 1998). Consequently, estimates of their spawning stock biomass in the north-west Atlantic Ocean decreased from 433 000 t in 1988 to a low of 26 700 t in 1998 (NEFSC, 2006). In general, shark populations are especially susceptible to overfishing due to their slow growth, long life span and low fecundity (Holden, 1973; Musick, 1999; Stevens *et al*., 2000). As such, increased fishing pressure has led to changes in population

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dynamics of fish species, including increased growth rate (Sminkey & Musick, 1995; Stevens & West, 1997; Walker, 1998), a decrease in maximum size and younger age and smaller size at maturity (Buxton, 1993; Rijnsdorp, 1993; Rowell, 1993). Fishing pressure has affected the life-history parameters of *S. acanthias*, by decreasing the maximum size and size at maturity of females (Sosebee, 2005). Because changes in life-history parameters can alter the effectiveness of management strategies, updated and accurate parameters are critical.

Accurate and precise age estimates provide a foundation from which other important life-history variables used in management, such as growth, maturity, fecundity and mortality, are built upon, making it one of the most influential biological variables (Campana, 2001). In elasmobranchs, the use of vertebral centra is the predominant means of assessing age and determining growth rates (Cailliet & Goldman, 2004). In contrast, all previous ageing studies of *S. acanthias* and recently separated *Squalus suckleyi* (Girard 1855) in the north Pacific Ocean have utilized the second dorsal-fin spine to obtain age estimates (Kaganovskaia, 1933; Holden & Meadows, 1962; Jensen, 1965; Ketchen, 1972, 1975; Jones & Geen, 1977; Nammack *et al.*, 1985; McFarlane & Beamish, 1987; Campana *et al.*, 2009). While the method of age estimates using second dorsal-fin spines has been validated using oxytetracycline and bomb-radiocarbon (McFarlane & Beamish, 1987; Campana *et al.*, 2006), the dorsal-fin spines are located on the external surface of *S. acanthias* and are subjected to environmental factors that may cause wear or breakage. Although a quantitative method has been developed to account for lost annuli (Ketchen, 1975) in ageing studies of squalid sharks, this technique may err in the direction of overestimation of age and underestimation of growth rate (Ketchen, 1975; Cailliet & Goldman, 2004). Because such errors could result in unreliable and inaccurate age estimates, the need to develop alternate methods of age determination is warranted. Thus, the objectives of the present study are (1) to develop a staining technique to visualize growth increments on vertebral centra of *S. acanthias,* (2) to compare precision of age estimates obtained between dorsal-fin spines and vertebrae, (3) to fit the length-at-age data into growth curves using both structures and (4) to determine which structure is more appropriate for age estimates in *S. acanthias*, based on precision, accuracy, goodness of fit of the growth curves and observed biological data.

## MATERIALS AND METHODS

### SAMPLING PROTOCOL

Monthly samples of male and female *S. acanthias*, subdivided into 5 cm increment size classes over the range from <35 to >100 cm fork length ($L_F$), were obtained in the Gulf of Maine (*c.* 42°–45° N; 66°–71° W) between July 2006 and June 2009 from commercial fishing vessels using either bottom trawl or gillnet. Additional samples were obtained from the NOAA–NMFS spring and autumn Bottom Trawl Surveys as well as hook-and-line capture during this period.

For each *S. acanthias* collected, the sex was recorded, along with mass ($M$, to the nearest 0·05 kg), stretch total length ($L_{ST}$, measured from the tip of the rostrum to the termination of the dorsal lobe of the caudal fin at its maximum extension) and $L_F$ (measured from the tip of the rostrum to the most noticeable fork in the caudal fin). All length measurements were taken over the body and measured to the nearest 5 mm. The second dorsal-fin spine and eight to 12 vertebrae anterior to the first dorsal-fin, including the vertebra connected to

the first dorsal-fin spine, were removed from each *S. acanthias* and frozen until processed. By using the first dorsal-fin spine as a landmark, it ensured that the vertebrae removed and aged were from the same region between individuals, thus reducing variability between the vertebrae used for age estimates (Officer *et al*., 1996).

## PREPARATION OF VERTEBRAE AND SPINES

### Sectioning and staining of vertebrae

After excess tissue was removed from both structures and the individual vertebral centra were freed from the column, each respective hard part was stored frozen until further analysis. The $L_{ST}$ (cm) and vertebral diameter were plotted to determine if vertebral growth was proportional to fish growth. A positive relationship indicates the appropriateness of this structure for age estimations for this species. Regressions were plotted by sex and compared statistically using ANCOVA. Sexes were pooled if no difference was found.

In order to elucidate the growth increments (which are defined as translucent and opaque band pairs in the vertebral centra), it was necessary to perform several procedures for each vertebra. Initially, the centra were sectioned using a Raytech Gem Saw (www.raytech-ind.com) with two diamond blades separated by a 0·4 mm spacer. Each centrum was sectioned through the focus along the sagittal plane, and the resulting bow-tie sections were then stained to enhance the growth increments. A variety of staining techniques were attempted, including alizarin red (LaMarca, 1966), violet blue (Johnson, 1979) and silver nitrate (Schwarz, 1983). While these techniques failed, a modified histological staining method based on Natanson *et al*. (2007) successfully produced visible growth increments on the vertebral centra.

Sectioned vertebrae were placed in tissue cassettes and decalcified with 100% RDO rapid decalcifying agent (www.rdo-apex.com) for varying times from 0·5 to 1·5 h depending on size, with larger vertebrae needing longer decalcification times. Constant movement of the solution was provided by a mixing plate and magnetic stir-bar. Afterwards, centra were rinsed with running tap water for 1 h before they were stored in 70% ethanol until staining. Sections were stained using Harris' modified haematoxylin and put into glycerine in preparation for mounting with Kaiser glycerine jelly (see Table I for procedural details). Stained sections were finally mounted on microscope slides in glycerine jelly, covered by a cover slip.

## GROWTH INCREMENTS IN VERTEBRAE AND SPINES

### First increment validation

The second dorsal-fin spines and vertebrae from full-term pups were examined to identify the location of the growth increments, if any, that were formed *in utero*. If present, these increments would be subtracted from the total reading of unworn spines and vertebrae of postnatal *S. acanthias* to obtain more accurate age estimations. Full-term pups, defined as pups >24 cm $L_{ST}$ with no yolk sac, were removed from uteri of females in autumn, near the suspected time of parturition. Vertebrae and second dorsal-fin spines were removed from each pup, cleaned, processed and examined using the aforementioned protocol. The diameter of vertebrae and base of spines were measured to the nearest 0·1 mm using vernier calipers. The number of increments, if any, on both structures was counted and the widths at the increments measured.

### Postnatal vertebrae

Upon completion of staining, each vertebra from postnatal *S. acanthias* was examined twice by two readers who had no prior knowledge of size, mass, sex or month of capture. Sections from each vertebra were viewed with a dissecting microscope using transmitted light under ×30 magnification, and the number of increments was counted. The vertebrae were then digitally photographed using a compound microscope with transmitted light under ×40 magnification. The primary reader assigned an age to 394 vertebrae, while the secondary reader assigned an age to a sub-set of 207 vertebrae.

TABLE I. *Squalus acanthias* vertebra staining procedure modified from Natanson *et al.* (2007)

| Step | Formula | Time (min) | Notes |
|---|---|---|---|
| 1 | 100% distilled water | 5 | |
| 2 | Harris haematoxylin | 10 | Sections should be checked to ensure proper staining. Time can be adjusted depending on staining strength. |
| 3 | Water rinse | Until clear | |
| 4 | Acid alcohol | 2 | Consists of 35% alcohol, 65% dH$_2$O and 6 drops of HCl per 100 ml. Sections should be checked to ensure proper de-staining. Time can be adjusted depending on staining strength. |
| 5 | Water rinse | 1 | Use agitation |
| 6 | Running water | 10 | |
| 7 | Distilled water | 2 | |
| 8 | 25% glycerine | 10 | |
| 9 | 50% glycerine | 10 | |
| 10 | 75% glycerine | 10 | |
| 11 | 100% glycerine | 10 | Tissues can be stored for longer periods at this step |

*Postnatal spines*

Whole second dorsal-fin spines were used for age estimations. The base of the spine at the beginning of enamel deposition was measured to the nearest 0·1 mm from anterior to posterior surfaces using vernier calipers. Spines were also aged twice by two readers who had no prior knowledge of size, mass, sex or month of capture of *S. acanthias*. Each reader counted the number of ridges and pigment bands on the anterior lateral surface of the spines using a dissecting microscope under ×30 magnification, beginning with the first ridge at the base that did not contain a white band. Incomplete bands at the base that were in the process of forming were white and not counted (Nammack *et al.*, 1985). If external wear of the enamel on the spine surface occurred, the increment number was counted up to the point of wear and the diameter of the spine from anterior to posterior face was measured with vernier calipers. Using the method developed by Ketchen (1975), a power curve was constructed using the width at the base of unworn spines and the number of increments observed by the readers. The width at the wear point of worn spines could then be put into the equation to account for lost increments, which were then added to the count obtained up to the wear point to give an adjusted age estimate. The primary reader assigned an age to 381 dorsal spines, while the secondary reader aged a random subset of 196 spines.

## AGE ANALYSIS OF VERTEBRAE AND SPINES

*Precision estimates and age bias*

Average per cent error (APE) and coefficient of variation (c.v.) (Chang, 1982) were calculated to determine the precision of the between and within reader counts for both spines and vertebrae. The average per cent error $= R^{-1} \sum_{i=1}^{R} \left( X_{ij} - X_j \right) X_j^{-1}$ and the coefficient of variation $= \sqrt{\sum_{i=1}^{R} \left( X_{ij} - X_j \right)^2 * (R-1)^{-1} X_j^{-1}}$, where $X_{ij}$ is the $i$th age determination of the $j$th fish, $X_j$ is the average age calculated for the $j$th fish and $R$ is the number of times each fish is aged. Contingency tables were analysed using methods devised by Bowker (1948) and McNemar (1947), and age bias plots (Campana, 2001) were used to expose any possible systematic bias in estimating age between readers.

*Marginal increment analysis*

Unlike dorsal-fin spines (McFarlane & Beamish, 1987; Campana *et al.*, 2006), vertebrae have not been validated as an ageing structure in squalid sharks. Marginal increment analysis was used to verify the time of increment formation and provided a validation of increment periodicity in vertebrae. Measurements were taken from all *S. acanthias* with age estimates >1 year following the protocol of Conrath *et al.* (2002). The margin width ($W_M$), which is the distance from the distal portion of the last opaque increment and the vertebra edge, as well as the previous band width ($W_{PB}$), between the distal portion of the next to last opaque increment and the distal portion of the last opaque increment, were recorded. The formula to determine standardized marginal increment ($M_s$) formation for each fish was $M_s = W_M \ W_{PB}^{-1}$. Mean $M_s$ were plotted by month to examine trends in increment formation. If increments occurred annually, there will be a unimodal distribution of the marginal increments throughout the year (Manooch, 1987). A multi-way ANOVA was performed to test for differences in standardized marginal increments throughout the year. A pair-wise comparison was then performed to determine time of year of the band formation.

## GROWTH DETERMINATION

Multiple growth curves were fitted to length-at-age estimates obtained from second dorsal-fin spines and stained vertebral centra from both males and females. Two versions of the von Bertalanffy growth function (VBGF; von Bertalanffy, 1938) were utilized, according to the equation: $L_t = L_\infty - (L_\infty - L_0) \ e^{-kt}$, where $L_t$ is the $L_{ST}$ at age $t$, $L_\infty$ is the theoretical mean maximum $L_{ST}$ in the population, $k$ is the growth coefficient and $L_0$ is the $L_{ST}$ at birth. Because size at birth was known, a two-parameter version of the VBGF with a fixed $L_0$ of 25 cm $L_{ST}$ was utilized. The Gompertz growth model (Gompertz, 1825) was a third model used according to the equation: $L_t = L_0 e^{G[1-e^{(-kt)}]}$, where $L_\infty$ is equal to $L_0 e^G$, which is the mean maximum $L_{ST}$ in the population ($t = \infty$), $k$ ($= g$ in Ricker, 1975) is the rate constant (per year) and $L_0$ is the $L_{ST}$ at birth.

Model fit was examined using several criteria, including Akaike's (1974) information criteria (AIC), comparisons between mean square error of the residuals (MSE) (Cailliet *et al.*, 2006), and $L_\infty$ and $L_0$ were compared to the observed maximum $L_{ST}$ and $L_{ST}$ at birth from individuals used in this study to determine if these parameter estimates were biologically realistic (Natanson *et al.*, 2006). While $L_\infty$ is not equivalent to maximum size, it does provide a rough estimate in which observed lengths can be compared. These three methods only determine fit of the model to those specific data, so comparisons cannot be made between sex and ageing structure, only model type.

## LONGEVITY

The maximum age estimates generated from vertebrae and second dorsal-fin spines provided an approximation of longevity for *S. acanthias*. In order, however, to account for a potential underestimation of maximum age obtained due to the lower frequency of fish at the extreme sizes, the method devised by Taylor (1958) to determine longevity based on VBGF parameters from observed data was utilized. Subsequently, Skomal & Natanson (2003) substituted $L_0$ for 99% of $L_\infty$ ($y$) to yield the following equation:

$$y = k^{-1} \ln\{(L_\infty - L_0)[L_\infty(1 - 0.99)]^{-1}\}.$$

## DETERMINING THE BETTER STRUCTURE FOR ESTIMATING AGE

The utility of vertebrae and second dorsal-fin spines as accurate ageing structures was evaluated by comparing precision estimates and growth models for each ageing structure. The growth models were statistically compared by ageing structure using a maximum likelihood ratio test (Kimura, 1980). If a statistically significant result was obtained, then individual

parameters were tested between structures following the methods of Kimura (1980). Further, age estimates obtained from the two structures in the same individuals were compared to quantify differences.

## RESULTS

Between July 2006 and June 2009, a total of 395 (147 males and 248 females) *S. acanthias* was collected during all months of the year except January. The $L_{ST}$ of males and females ranged from 32·5 to 84.0 and 25.0 to 102.0 cm. There was a strong correlation ($n = 368$; $r^2 = 0·99$) between $L_F$ and $L_{ST}$ described by the equation: $L_F = (L_{ST} - 2.1572\times)\ 1.0939$.

In addition, there was a positive logarithmic relationship between $L_{ST}$ and vertebrae diameter ($n = 90$, $r^2 = 0·94$), with no significant difference between sexes (ANCOVA; d.f. $= 1$, $P > 0·05$; Fig. 1). Because vertebral diameter was correlated strongly with $L_{ST}$, it is an appropriate structure for age determination. Further, the modified histological staining method utilized for this study also enhanced the visibility of the growth increments in the vertebrae (Fig. 2). Notably, the decalcification step was critical, since growth increment visualization was not enhanced in vertebrae stained with haematoxylin before decalcification.

### BIRTH-RING CONFIRMATION

The birth ring on postnatal *S. acanthias* vertebrae was confirmed because the mean diameter of the putative birth ring was not significantly different from the vertebral diameter of full-term embryos, and they were both significantly smaller than the vertebral diameter at the first increment of postnatal *S. acanthias* (ANOVA, d.f. 2, 111, $P < 0·001$) (Table II).
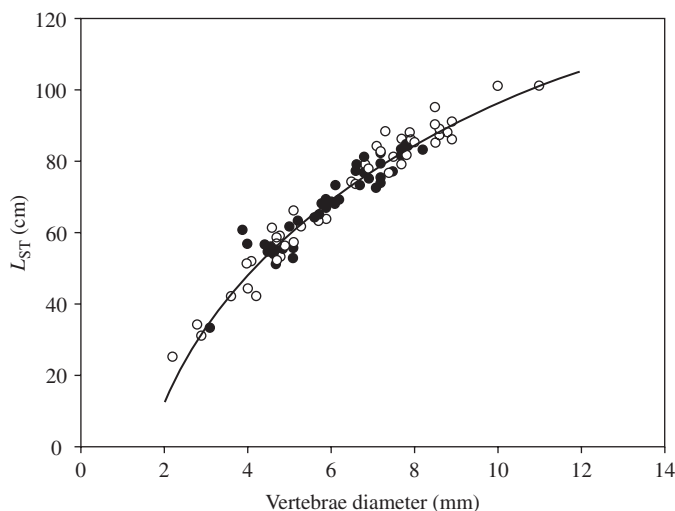


FIG. 1. Vertebrae diameter (mm) as a function of total stretched lengths ($L_{ST}$) in male (●) and female (○) *Squalus acanthias*. The curve was fitted to the data by: $y = 51·72 \ln x - 23·23$.
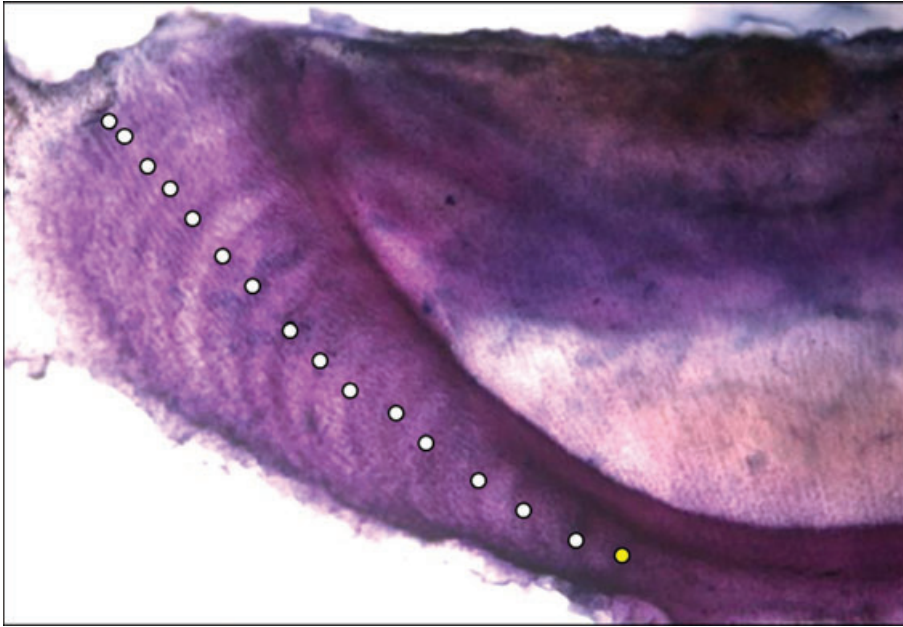
FIG. 2. A representative sectioned vertebra of a mature male *Squalus acanthias* prepared according to the modified staining method. Opaque increments of each band pair are marked (○), with the birth band indicated (●).

Similarly, the birth ring on postnatal *S. acanthias* second dorsal-fin spines was confirmed because the mean diameter of the putative birth ring on the postnatal dorsal-fin spine and the spine diameter of full-term embryos were significantly smaller than the spine diameter at the first increment of postnatal *S. acanthias* (ANOVA, d.f. 2, 366, $P < 0.001$) (Table II).

## VALIDATION OF GROWTH INCREMENTS

Marginal increment analysis of vertebrae from all *S. acanthias* $\geq 1$ year of age displayed a statistically significant unimodal distribution across all months (Kruskal–Wallis, d.f. $= 10$, $P < 0.001$), suggesting that the band pairs were deposited annually

TABLE II. Mean $\pm$ S.D. diameters of dorsal-fin spines and vertebral centra for full-term embryos and birth ring and first increments of both structures for postnatal *Squalus acanthias*. Significant differences ($P < 0.05$) within each structure are denoted by different superscript lower case letters

|  | $n$ | Spine (mm) | Range (mm) | $n$ | Vertebra (mm) | Range (mm) |
|---|---|---|---|---|---|---|
| Full-term embryo | 15 | $1.61 \pm 0.18^a$ | 1.30–2.00 | 15 | $0.98 \pm 0.11^a$ | 0.80–1.20 |
| Birth ring | 184 | $1.72 \pm 0.11^b$ | 1.40–2.00 | 50 | $1.07 \pm 0.08^a$ | 0.90–1.20 |
| First increment | 184 | $2.20 \pm 0.15^c$ | 1.90–2.60 | 50 | $1.50 \pm 0.13^b$ | 1.30–1.80 |

n, sample size.

with the beginning of translucent band formation occurring between April and July (Fig. 3). Ideally, each year class would be validated (Campana, 2001), but the logistics of obtaining adequate animal numbers and representative sizes for each month were unrealistic.

*Bias and precision*

Minimal bias was possible in bias plots for either structure at the oldest ages (Fig. 4), but contingency table analyses from age estimates using vertebrae (Bowker's and McNemar's, $n = 206$; $P > 0.05$) and dorsal-fin spines (Bowker's and McNemar's, $n = 195$; $P > 0.05$) showed no bias. The initial agreement on age estimates was 43·9% (90·1% within 2 years) using vertebrae, while the initial agreement between readers using dorsal-fin spines was 30·4% (78·9% within 2 years). A significantly higher degree of inter-reader (Wilcoxon signed ranks test, $n = 196$, $P < 0.05$) and intra-reader (Wilcoxon signed ranks test, reader 1: $n = 378$; $P < 0.001$; reader 2: $n = 196$, $P < 0.001$) precision was observed in age estimates using vertebrae rather than second dorsal-fin spines, as indicated by lower c.v. and APE values (Table III).

## WEAR OF SECOND DORSAL-FIN SPINE

Of all the second dorsal-fin spines taken for age estimates, 53% had wear to a diameter greater than the mean diameter measured for the first increment on unworn spines, with the oldest age estimate obtained from an unworn spine being 13 years.
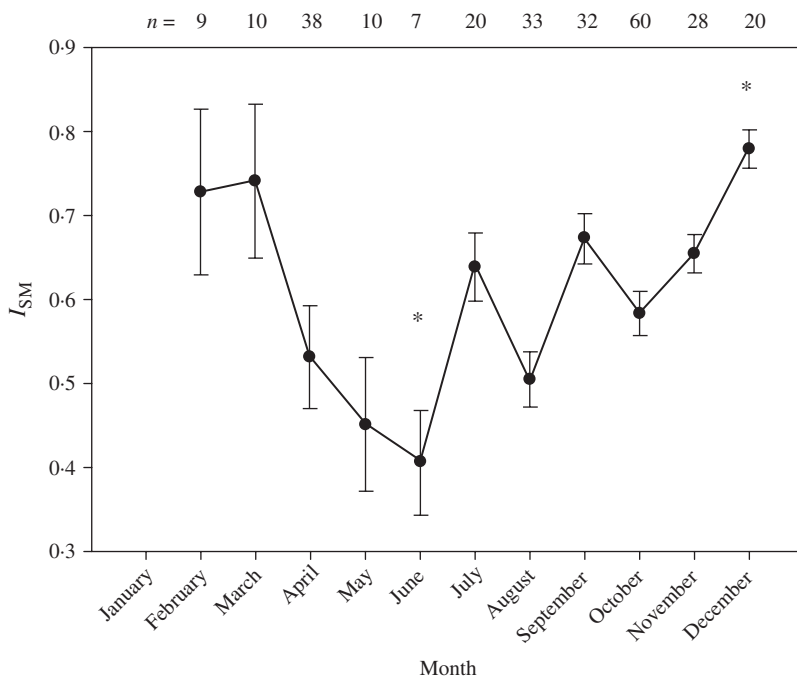


FIG. 3. Mean ± s.e. standardized marginal increment ($I_{SM}$; ●) by month on measurements of vertebrae taken from *Squalus acanthias*. ∗ Months are significantly different ($P < 0.05$) from each other. $n$, sample size.
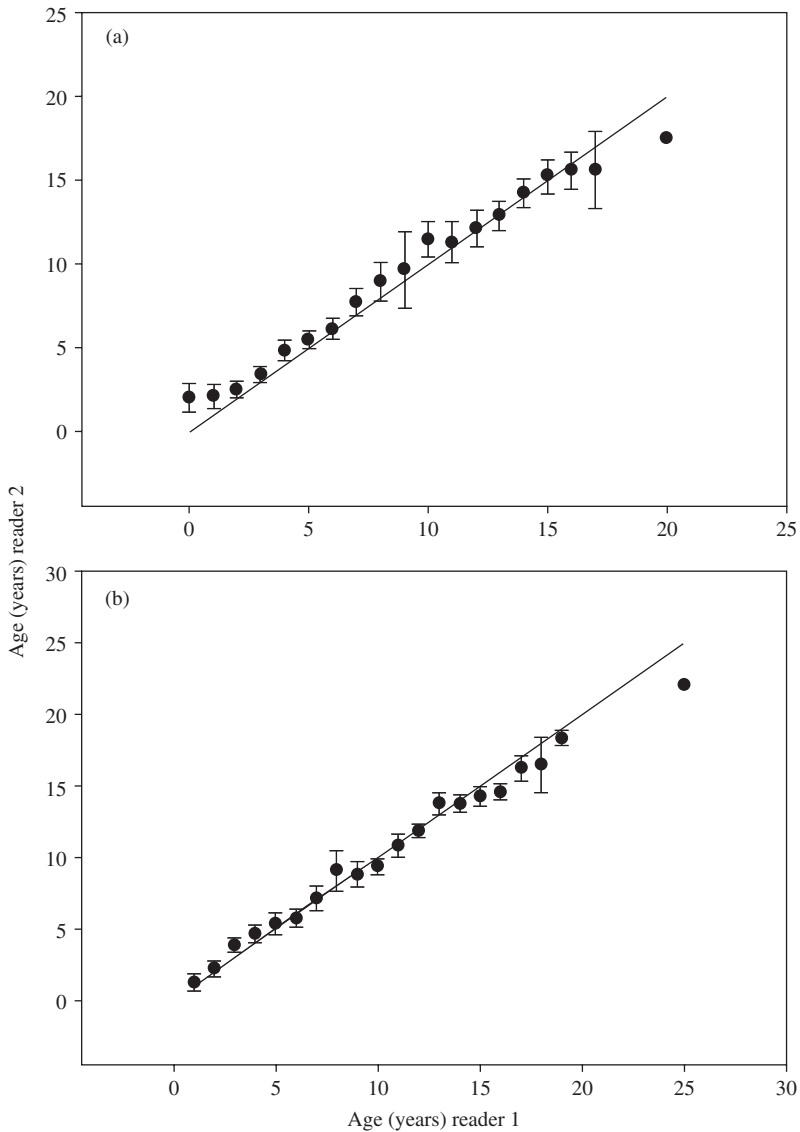
FIG. 4. Bias plot between the primary (1) and secondary (2) readers of age estimates of *Squalus acanthias* (●) made using (a) second dorsal-fin spines and (b) vertebrae. The primary and secondary readers read 394 and 207 second dorsal-fin spines and 381 and 196 vertebrae, respectively. Values are mean ± 2 S.E.

The width-at-wear point increased with $L_{ST}$ of *S. acanthias* (ANOVA, d.f. = 1, 324, $P < 0.001$), with the mean diameter of the wear point on spines = 3·13 mm. In 132 *S. acanthias* that were <60 cm $L_{ST}$, 6% had a wear point on the spine which would have eliminated increments, while in 249 *S. acanthias* >60 cm $L_{ST}$, that number increased to 76%. The equation to account for lost increments due to wear of the second dorsal-fin spine (Ketchen, 1975) was created by plotting the width of the base of 174 unworn spines and the estimated age obtained by the readers (Fig. 5).

TABLE III. Precision estimates of within (intra) and between (inter) reader variation using average per cent error (APE) and coefficient of variation (C.V.). All statistical comparisons of variation are between the two ageing structures (vertebra and second dorsal-fin spine). Statistical significance ($P < 0.05$) using Kruskal–Wallis signed rank test was between values bearing the same superscript symbol; [x,xx,xxx,†,††or‡]

|  | Structure | APE | S.E. | C.V. | S.E. | $n$ |
|---|---|---|---|---|---|---|
| Intra-reader variation: reader 1 | Vertebra | 4·79[x] | 0·44 | 6·77[†] | 0·63 | 378 |
|  | Dorsal-fin spine | 7·40[x] | 0·45 | 10·47[†] | 0·64 |  |
| Intra-reader variation: reader 2 | Vertebra | 6·30[xx] | 0·56 | 8·90[††] | 0·80 | 196 |
|  | Dorsal-fin spine | 8·75[xx] | 0·58 | 12·37[††] | 0·82 |  |
| Inter-reader variation | Vertebra | 6·23[xxx] | 0·66 | 8·81[‡] | 0·94 | 196 |
|  | Dorsal-fin spine | 8·82[xxx] | 0·68 | 12·48[‡] | 0·96 |  |

## DIFFERENCES BETWEEN AGEING STRUCTURES

Mean vertebrae-derived age estimates of *S. acanthias* (mean ± S.D. = 9·2 ± 4·9 years; range 0–24 years) were significantly lower (ANOVA, d.f. = 1, 383, $P <$ 0·001) than the estimated ages derived from the second dorsal-fin spines including those corrected for wear (mean ± S.D. = 11·2 ± 6·0 years, range 0–28 years). The spine age estimates were then further divided into those that were corrected for wear and those that were unworn. In individuals >60 cm $L_{ST}$, in which spines were corrected for wear, mean ± S.D. vertebral derived age estimates (13·0 ± 2·90 years) and mean ± S.D. age estimates from dorsal spines (16·3 ± 3·5 years) were significantly different from each other (Wilcoxon – Kruskal–Wallis, $n = 184$, $P < 0.001$).
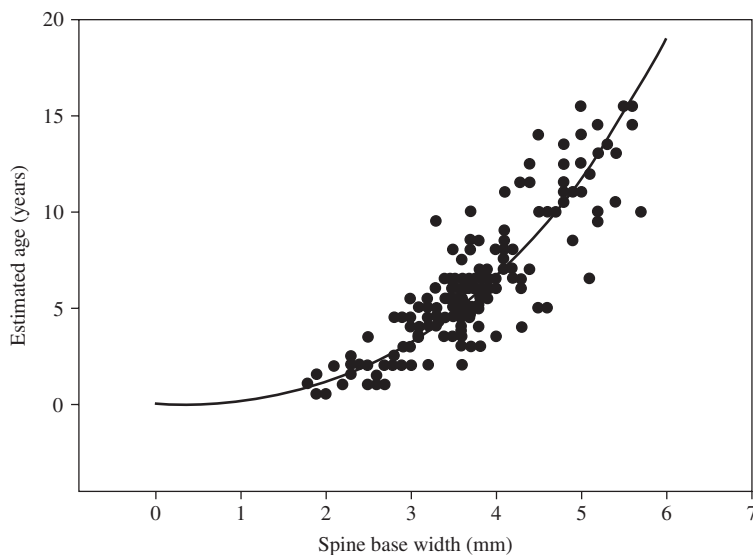


FIG. 5. Best fit line of spine base width and estimated age (●) using *Squalus acanthias* unworn second dorsal-fin spines ($n = 174$) to account for lost increments in worn spines (Ketchen, 1975). The curve was fitted by $y = 0.1808\, x^{2.5979}$.

Interestingly, mean age estimates using vertebrae (mean $\pm$ s.d. $= 9 \cdot 0 \pm 2 \cdot 7$ years) and unworn spines (mean $\pm$ s.d. $= 9 \cdot 7 \pm 3 \cdot 1$ years) from *S. acanthias* $> 60$ cm $L_{ST}$ were not significantly different (Wilcoxon – Kruskal–Wallis, $n = 56$, $P > 0 \cdot 05$). This difference was apparent when worn and unworn spines were plotted against the corresponding age estimates obtained using vertebrae (Fig. 6). This illustrated that Ketchen's equation increases the age and thus decreases the mean size-at-age.

GROWTH-FUNCTION ANALYSIS

All growth curves had similar fit to length-at-age data generated from second dorsal-fin spines and vertebrae of both sexes (Table IV), with no statistically significant differences between the fit of the models (ANOVA, d.f. = 3, 855, $P > 0 \cdot 05$). The Gompertz model produced the lowest $L_\infty$ values and conversely yielded the highest $k$ values for both sexes and ageing structures and had some of the lowest
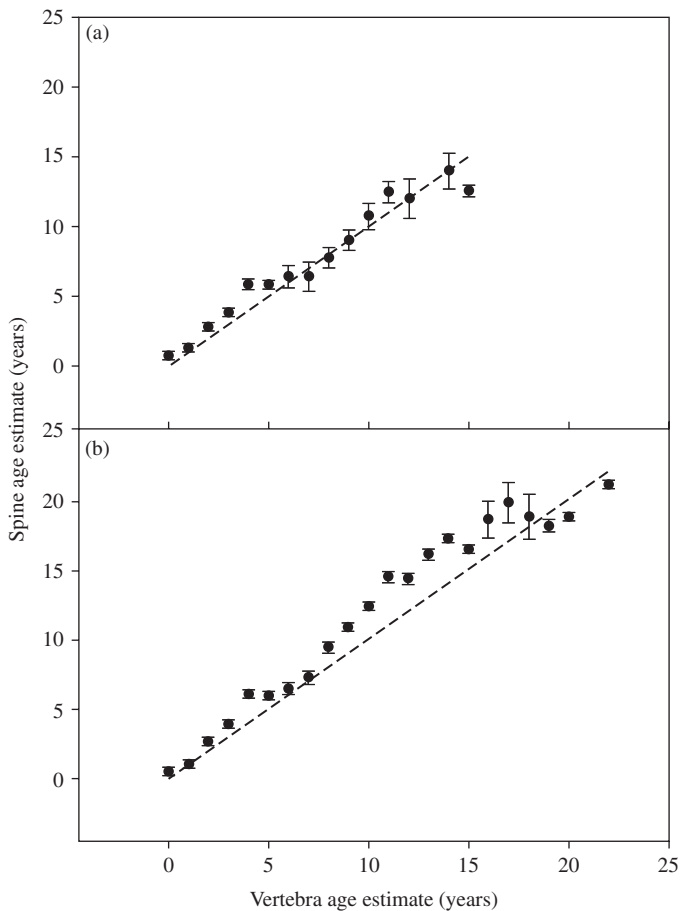


FIG. 6. Mean $\pm$ s.e. age estimates (●) of *Squalus acanthias* obtained using (a) unworn and (b) worn dorsal-fin spines corrected to account for lost increments. The one to one line between vertebrae and dorsal-fin spine estimates is included (_ _ _ _).

TABLE IV. Growth parameters, goodness of fit [mean square of errors (MSE)] and Akaike Information Criteria (AIC) values by sex using vertebrae and second dorsal-fin spines from *Squalus acanthias*. Two and three-parameter (par) von Bertalanffy (VB) and Gompertz growth function mean maximum total stretch length ($L_\infty$), growth rate constant ($k$) and total stretch length at birth ($L_0$) are shown with 95% c.i. The two-parameter models had a fixed $L_0$ (25 cm) at the age of birth determined in the present study

| Sex | Ageing structure | Growth function | $L_\infty$ | $k$ | $L_0$ | MSE | $n$ | AIC |
|---|---|---|---|---|---|---|---|---|
| Female | Vertebra | VB 2 par | 100.76 ± 16.27 | 0.12 ± 0.02 | 25.00 | 39.0 | 239 | 615 |
| Female | Vertebra | VB 3 par | 99.89 ± 16.07 | 0.13 ± 0.02 | 23.90 ± 3.78 | 39.0 | 239 | 615 |
| Female | Vertebra | Gompertz 2 par | 95.30 ± 12.77 | 0.20 ± 0.03 | 25.00 | 37.9 | 239 | 611 |
| Female | Vertebra | Gompertz 3 par | 97.21 ± 13.02 | 0.18 ± 0.02 | 28.10 ± 3.76 | 38.4 | 239 | 610 |
| Female | Spine | VB 2 par | 107.17 ± 17.71 | 0.08 ± 0.01 | 25.00 | 37.9 | 224 | 589 |
| Female | Spine | VB 3 par | 117.54 ± 17.72 | 0.06 ± 0.01 | 30.50 ± 4.09 | 39.4 | 224 | 597 |
| Female | Spine | Gompertz 2 par | 96.28 ± 12.90 | 0.15 ± 0.02 | 25.00 | 39.8 | 224 | 598 |
| Female | Spine | Gompertz 3 par | 102.72 ± 13.76 | 0.11 ± 0.01 | 31.70 ± 4.25 | 36.4 | 224 | 598 |
| Male | Vertebra | VB 2 par | 94.23 ± 14.96 | 0.11 ± 0.02 | 25.00 | 22.6 | 146 | 400 |
| Male | Vertebra | VB 3 par | 104.98 ± 16.83 | 0.08 ± 0.01 | 29.50 ± 4.97 | 21.8 | 146 | 399 |
| Male | Vertebra | Gompertz 2 par | 84.68 ± 13.83 | 0.20 ± 0.03 | 25.00 | 21.5 | 146 | 400 |
| Male | Vertebra | Gompertz 3 par | 93.63 ± 15.29 | 0.14 ± 0.02 | 31.10 ± 5.08 | 23.8 | 146 | 399 |
| Male | Spine | VB 2 par | 91.46 ± 14.36 | 0.11 ± 0.02 | 25.00 | 21.3 | 144 | 380 |
| Male | Spine | VB 3 par | 121.82 ± 14.48 | 0.05 ± 0.02 | 35.50 ± 4.21 | 20.9 | 144 | 376 |
| Male | Spine | Gompertz 2 par | 83.67 ± 13.67 | 0.18 ± 0.03 | 25.00 | 21.6 | 144 | 384 |
| Male | Spine | Gompertz 3 par | 99.10 ± 16.19 | 0.10 ± 0.02 | 35.50 ± 5.80 | 20.3 | 144 | 375 |

AIC scores. While the fit using MSE and AIC was comparable to the VBGFs, the Gompertz growth model did not provide biologically realistic results for $L_0$ and so was excluded from further analysis.

The two and three-parameter VBGF provided more realistic values of size at birth and maximum size for both sexes, using both ageing structures. While the three-parameter VBGF allowed for comparisons between the observed size at birth and the predicted size at birth, the two-parameter VBGF did not because $L_0$ was fixed. Further, the three-parameter VBGF fit to vertebral derived age estimates produced values closer to the known size at birth than those obtained using dorsal-fin spines. The $L_\infty$ values obtained from the two and three-parameter VBGF using both ageing structures for males (range $= 91.5-105$ cm $L_{ST}$) and females (range $= 95.3-117.5$ cm $L_{ST}$) were in the appropriate range based on the observed data. The exception, however, was the three-parameter VBGF using male dorsal-fin spines, which had a value that was 45% higher than the largest observed male *S. acanthias*, making it much higher than would be expected. Although both the two and three-parameter VBGFs provided similar results, the two-parameter VBGF was the more appropriate and provided the better biological fit for both sexes because the size at birth is known for *S. acanthias*. Overall, the growth models suggest that males attain a smaller $L_\infty$ than females (Fig. 7). The growth models also are significantly different between sexes (maximum likelihood ratio test, $n = 380$, $P > 0.05$) being attributed to differences in both $L_\infty$ (maximum likelihood ratio test, $n = 380$, $P > 0.05$) and $k$ (maximum likelihood ratio test, $n = 380$, $P > 0.05$). The observed size at age between sexes did not differ until the age of 10 years (ANOVA, d.f. $= 1, 21$, $P < 0.001$).

There were no statistically significant differences between the growth curves produced from age estimates obtained from dorsal-fin spines and vertebrae in male *S. acanthias* (maximum likelihood ratio test, $n = 144$, $P > 0.05$). Conversely,
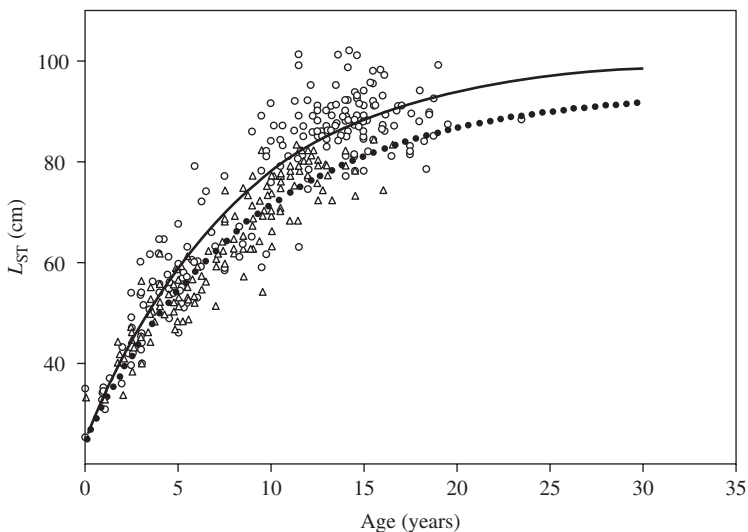


FIG. 7. Two-parameter von Bertalanffy growth functions [in stretched total length ($L_{ST}$)] of females (———) and males (......) of *Squalus acanthias* based on age estimates for females (O) and males (△) obtained from sectioned and stained vertebrae.

TABLE V. Longevity estimates obtained from vertebrae and dorsal-fin spines of female and male *Squalus acanthias.* The oldest observed values obtained by sex and structure are listed. Values for Taylor (1958) (99%) method of longevity estimates were calculated by using $L_\infty$ and $k$ values of a two-parameter von Bertalanffy growth function

| Sex | Ageing structure | Oldest age observed (years) | Taylor 99% |
|---|---|---|---|
| Female | Vertebra | 24 | 35·8 |
| Female | Spine | 28 | 53·4 |
| Male | Vertebra | 17 | 38·6 |
| Male | Spine | 22 | 40·6 |

growth curves developed using age estimates from both structures differed significantly in female *S. acanthias* (maximum likelihood ratio test, $n = 214$, $P < 0.001$). The differences could be attributed to changes in both $L_\infty$ (maximum likelihood ratio test, $n = 214$, $P < 0.001$) and $k$ (maximum likelihood ratio test, $n = 214$, $P < 0.001$).

## LONGEVITY ESTIMATES

The oldest male and female *S. acanthias,* based on age estimates using vertebral centra, were 17 and 24 years old. On the other hand, the oldest age estimates using dorsal-fin spines for male and female *S. acanthias* were 22 and 28 years old. It should be noted that the oldest estimated age for females was a result of using Ketchen's equation to account for an almost completely worn spine. Overall, longevity estimates based on Taylor's calculations (1958) were similar between sexes when using vertebral centra (Table V). They differed, however, by 13 years when second dorsal spines were used, with the females reaching an older maximum age than males. For both sexes, longevity estimates obtained using growth curves derived from vertebrae age estimates were lower than those derived from dorsal-fin spines (Table V).

## DISCUSSION

Age information forms the basis for the calculations of growth rate, mortality rate and productivity, making it one of the most important variables for estimating a population's status and assessing the risks associated with exploitation. Since difficulty in enumerating opaque and translucent bands in the vertebrae exists in *S. acanthias*, previous ageing studies, including the most recent studies in the western North Atlantic Ocean (Nammack *et al*., 1985; Campana *et al*., 2009), have utilized the second dorsal-fin spine. Large variability, however, exists in age estimates among studies that have utilized this ageing structure in *S. acanthias*. For example, two studies in the Atlantic Ocean, published 3 years apart, that used second dorsal-fin spines as the structure to estimate age had maximum ages varying from 21 to 40 years old for females (Soldat, 1982; Nammack *et al*., 1985).

Research by Ketchen (1975) and personal observations suggest that the damage to the dorsal spine is correlated to the age and size of the individual, *i.e*. dorsal spines

of older and larger *S. acanthias* have more wear and breakage than younger and smaller ones. In order to compensate for spine damage and wear, Ketchen (1975) developed an equation that compensated for the lost annuli, based on spine base width. The information presented in the current manuscript suggests that vertebral centra provide a more precise means of estimating the age of *S. acanthias* when compared to age estimates obtained from dorsal-fin spines, especially in larger and older individuals for which wear and breakage must be accounted for. For example, when the equation developed from the spine base width of unworn samples for the present study was examined, dorsal spines corrected for substantial wear had significantly higher age estimates than those obtained from vertebrae. In contrast, age estimates obtained for large unworn spines were not significantly different from those obtained from the vertebrae for the same individuals, which suggests that the equation developed by Ketchen (1975) produced overestimates in the number of increments missing from worn spines. In addition to the error associated with the use of the equation itself to account for lost increments due to wear, there is also an error associated with the interpretation and measurement of the wear point on the spine (McFarlane & King, 2009). For example, McFarlane & King (2009) determined that errors in measuring the wear point to adjust for missing annuli can cause differences from 1 to 19 years between readers examining the same spine in *S. suckleyi* (as *S. acanthias*). Thus, by utilizing an ageing structure such as the vertebral centrum, which is internal and not subject to environmental wear or breakage, these sources of error can be eliminated, which ultimately leads to increased precision and accuracy of age estimates.

In order to use the vertebral centrum as an ageing structure in *S. acanthias,* developing a technique to visualize the growth increments as well as verifying the structure as being appropriate to obtain consistent age estimates and validation of the periodicity of increment deposition were required. This was accomplished by modifying the histological staining technique of Natanson *et al.* (2007). Based on the strong correlation among $L_{ST}$ and vertebra diameter, the location of the birth band and first increment, marginal increment analysis suggesting annual increment deposition and the relatively low APE and c.v. values (Campana, 2001; Jackson *et al.*, 2007; Sharma & Borgstrom, 2007), vertebrae were deemed an appropriate ageing structure.

Although the two and three-parameter VBGF and Gompertz growth functions were examined in the present study, the two-parameter VBGF was preferred because the size at birth of *S. acanthias* was known, *i.e.* $L_0$ was fixed. The growth curves generated from $L_{ST}$-at-age data using both ageing structures for males and females provided a good fit, based on the mean square of errors and AIC. Further, age estimations from both ageing structures produced growth curves in which the $L_\infty$ parameter was realistic, but the three-parameter growth curves using vertebrae were closer in approximating parameters for $L_0$, which was known based on the observed data obtained from the present study. Thus, the two-parameter VBGF using age estimates obtained from vertebral centra provided the most realistic fit to length and age data in this species and should be used for future management purposes.

The growth curves fit to age estimates using dorsal-fin spines and vertebrae were significantly different for female *S. acanthias*, with the $L_\infty$ being higher and the $k$ value being lower for ages obtained using dorsal-fin spines. This is consistent with the observation of an overestimation of age using dorsal-fin spines due to methods used to account for lost increments caused by wear or breakage. This trend was not

present in the males, possibly due to the shorter life span and less need to account for missing increments.

Von Bertalanffy parameter estimates obtained in the present study had similarities and differences from other studies conducted in the western North Atlantic Ocean (Nammack *et al.*, 1985; Campana *et al.*, 2009) (Table VI). While there appears to be a correlation between some of the observed changes and increased fishing pressure, it must be noted that these differences could be affected by any number of other factors, including but not limited to, spatial and temporal differences in sampling as well as sample size and representation of all size classes. Thus, caution should be taken when drawing conclusions regarding population-level changes in life-history variables. For example, the growth parameters for males were closer between the three studies, having similar $L_\infty$ and $k$ values, possibly due to males not being targeted in the fishery. In contrast, while the females from the present study and those obtained by Nammack *et al.* (1985) have similar $L_\infty$ and $k$ values, these values were notably different than those obtained by Campana *et al.* (2009). It must be noted, however, that parameter estimates obtained using dorsal-fin spines for females in the present study, showed a slight decrease in $k$ compared to Nammack *et al.* (1985). The decreased growth rate could be a result of the fishery selecting against the faster growing and thus larger fish (Law, 2000). With the size-selective nature and the magnitude of the biomass decline following fishing pressure, larger size and growth differences would be expected between females from the present study and that by Nammack *et al.* (1985) (Sminkey & Musick, 1995; Stevens & West, 1997). One possible explanation provided by Sosebee (2005) was that shortly preceding the study by Nammack *et al.* (1985), *S. acanthias* faced heavy commercial fishing pressure from foreign vessels, which may have already caused a decrease in size. Following

TABLE VI. Comparison of published values of von Bertalanffy growth function (VBGF) parameters and longevity of *Squalus acanthias* with those obtained from the present study. Longevity was calculated from the VBGF parameters shown using the method devised from Taylor (1958) and modified by Skomal & Natanson (2003) to give age at 99% of $L_\infty$

| Study | Present | | Nammack *et al*. (1985) | Campana *et al*. (2009) |
|---|---|---|---|---|
| Location | North-east U.S.A. | North-east U.S.A. | North-east U.S.A. | Eastern Canada |
| Structure | Vertebrae | Spines | Spines | Spines |
| Male | | | | |
| $L_\infty$ | 94·23 | 91·46 | 82·49 | 88·12 |
| $k$ | 0·110 | 0·106 | 0·148 | 0·099 |
| Female | | | | |
| $L_\infty$ | 100·76 | 107·17 | 100·50 | 133·35 |
| $k$ | 0·121 | 0·081 | 0·107 | 0·042 |
| Male and Female | | | | |
| Observed longevity | 24 | 28 | 40 | 31 |
| Calculated longevity | 38·6 | 53·4 | 40·5 | 104 |
| $L_{ST}$ at birth (cm) | 25 | 25 | 24·9 | 30·35 |

$L_{ST}$, stretched total length.

the elimination of foreign fishing pressure, size and maturity estimates began to a trend upwards (Marques da Silva, 1993) due to lowered landings of the species by U.S. commercial vessels (Rago *et al*., 1998). Thus, even though the Nammack *et al*. (1985) study was conducted prior to the most recent increase in fishing pressure and the present study, both were conducted during a time of biomass increase.

Perhaps the largest discrepancy among Nammack *et al*. (1985), Campana *et al*. (2009) and the present study in terms of the growth curves is the calculation of theoretical longevity. Although the oldest observed ages for *S. acanthias* from these three studies differed by only 12 years, the range of the calculated longevities based on Taylor (1958) was considerably larger (65·4 years). It is important to note, however, that this marked discrepancy is largely a result of the longevity estimate of 104 years, based on growth parameters, as reported by Campana *et al*. (2009). This age estimate would suggest that *S. acanthias* in Canadian waters of the western North Atlantic Ocean are slower growing and longer lived than *S. suckleyi* (as *S. acanthias*) in the North Pacific Ocean (Ketchen, 1975). This finding is in contrast, however, to an earlier report (Campana *et al*., 2006), which showed that *S. suckleyi* in the North Pacific Ocean attain older ages than *S. acanthias* in the western Atlantic Ocean, based on bomb radiocarbon dating. Interestingly, the age estimates obtained from dorsal-fin spines during the present study are consistent with the validated spine increments using bomb radiocarbon of *S. acanthias* from the western North Atlantic Ocean (Campana *et al*., 2006). Thus, at the present time, there is insufficient information to explain the causes of such large differences between the aforementioned studies, although they could be due to such variables as sample size, population differentiation (Campana *et al*., 2009) or errors inherent with using an external structure in the age estimates (Ketchen, 1975; McFarlane & King, 2009).

In conclusion, the findings of the present study indicate that the vertebra, processed with the modified histological staining technique, was a more appropriate structure for estimating the age of *S. acanthias* than the traditional structure, the second dorsal-fin spine, due to elimination of introduced error to account for wear, high precision of the age estimates and the good fit and realistic parameters of the growth curves obtained from these estimates. These factors, in combination with the collection of samples following increased fishing pressure, make the ages obtained using vertebrae in the present study more biologically relevant to current management of *S. acanthias* in Atlantic waters off the U. S. A. than previous studies utilizing second dorsal-fin spines. Overall, the age estimates presented in the current manuscript will lead to less error propagation in fisheries models and more accurate predictions leading to sustainable management (Lai & Gunderson, 1987; Rivard & Foy, 1987) of this species. Further, the verification of the vertebra as an appropriate ageing structure using the modified histological staining technique described for *S. acanthias* not only affords more direct comparisons of age estimates between studies that use vertebra as the ageing structure but also the technique may be used on vertebrae obtained from hard-to-age species.

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