Working Paper: Updated age and growth estimates for spiny dogfish *Squalus acanthias*

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Abstract

This working paper describes updated age estimates and growth models for spiny dogfish *Squalus acanthias* obtained as part of the 2022 spiny dogfish research track assessment. Due to uncertainty in resulting ages and substantial differences in growth model parameters relative to those used in previous assessments, these ages were considered for sensitivity analyses only.

Introduction

The objective of this work is to provide updated length-at-age data and growth models for spiny dogfish *Squalus acanthias* per the research recommendations put forth by the 2020 SSC and 2019 MAFMC. Age determination in spiny dogfish has historically been a challenge due to their life history characteristics of being slow growing and long lived (maximum estimated age of 80+ years; McFarlane & King 2009), and because their use of deep water habitats generally results in a reduced ability to use calcified tissues such as vertebrae for age estimation (e.g. Campana et al. 2006). For these reasons, second dorsal fin spines have been widely used to age *S. acanthias* and other similar dogfish species based upon counts of growth bands formed in the external enamel of spines (e.g. Ketchen 1975, Beamish & McFarlane 1985, Tribuzio et al. 2010). While some research has suggested that vertebrae can be useful for ageing (e.g. Bubley et al. 2012), a radiocarbon validation study by Campana et al. (2006) showed spine-based ages to be accurate within 1-2 years relative to radiocarbon references, and a more recent mark-recapture study by James et al. (2021) showed that second dorsal fin spines are more reliable than vertebrae in terms of growth band deposition.

While these studies have demonstrated that spines can be used to age dogfish, nonetheless it remains a challenging method due to several factors, including hard-to-discern banding patterns as well as the loss of enamel in spines of older fish due to wear. This enamel loss precludes accurate growth band counts for older fish, and requires calculation and application of a correction factor to predict missing growth bands based on the spine diameter at the 'wear point' of worn spines relative to spine base diameter-at-age inferred from unworn spines (Ketchen 1975). This correction method has been used widely in age estimation studies for dogfish since (e.g., Campana et al. 2009, Tribuzio et al. 2017, Nammack 1985), but has well-established caveats including potential over-estimation of ages in worn spines as well as a lack of specificity for predicting differential growth across sexes and life stages because unworn spines tend to come from juvenile fish (i.e. Taylor et al. 2013). Despite these caveats, the lack of a better alternative for ageing these fish has led to spine-based ages being the 'best available' for stock assessment (e.g. Gerteseva et al. 2021), but high levels of uncertainty in estimates have led some assessments to use length-based estimates of population dynamics instead (e.g. NEFSC 2006).

Recent assessments of the Atlantic *S. acanthias* population have suggested overfishing, especially of the mature female population, truncation of the size/age distribution, reduced size at maturity, and reduced size at birth (e.g., NEFSC 2006). As such, it is desirable to reassess the age structure of Atlantic spiny dogfish for greater understanding of stock status.

Methods

Second dorsal fin spines were collected as part of two NOAA Fisheries scientific surveys between the years of 2006-2014. The majority of samples were sourced from the NEFSC spring and autumn bottom trawl survey between years 2006-2010 as described in Politis et al. (2014). The rest were collected as part of a mark-recapture study between 2011-2014 detailed in James et al (2021). Spines were cleaned of most external adhering tissue and stored dry in coin envelopes until analysis. Growth band counts and spine measurements were carried out by the Washington Department of Fisheries and Wildlife, Olympia, WA. Prior to counting bands, spines were inspected and distinguished as being either unworn or worn based on whether there was visual wear of enamel toward the tip of the spine. Worn spines had the 'wear point' marked with a marker. Any spines that were broken or otherwise damaged were not used in age analyses. Spine diameter measurements (to the nearest 0.01mm) were taken with calipers at the base of each spine as well as at the wear point, where applicable, in order to facilitate calculation of the worn spine correction factor as outlined by Ketchen (1975). As an additional calibration exercise, age readers, prior to band counting, reviewed images of age-validated spines from Campana (2006) and compared counts to validated ages. After this calibration, growth bands were visually enumerated a single time along the exterior surface of the spines under 10X magnification using a dissecting scope based on criteria outlined in Tribuzio et al. (2016). Spines were assigned a readability code of 1-6 based on clarity of annuli and condition of the spine (1=excellent, clear pattern, 2=average structure, 3=difficult structure, 4=minimum age only, difficult to age, 5=not aged, structure not discernable, 6=not aged, process or collectors error). Only spines with readability codes of 1-3 were included in analyses. A subset of spines were counted a second time by the same reader in order to generate precision and error metrics.

For spines exhibiting worn enamel, growth bands were counted up to the point of wear and a correction factor was calculated and used to predict the number of missing bands (Age_C) for each worn spine. This calculation was based on correlation of spine base diameter at age for unworn spines, to which a power function was fitted per Ketchen (1975). From this, Age_C was calculated for worn spines based on the spine diameter at the wear point (D):

$$Age_C = 0.2339 * D^{2.77} \quad (1)$$

Age_c was then added to annuli counts to calculate the final corrected ages for worn spines. In addition to spines with no visible wear, worn spines with a wear point of < 1.3mm, corresponding to the smallest recorded spine diameter with a detectable growth band from our samples, were considered unworn, corroborated by spine size at birth as reported by Nammack et al. (1985). Corrected ages were rounded to the nearest whole number before further analysis.

Data analysis

Length-at-age data were fitted to three different growth models. Von Bertalanffy growth functions (VBGF) were fitted using the original equation of von Bertalanffy (1938):

$$L_{(t)} = L_{\infty}\,(1^{-k(t-t_0)}) \qquad (2)$$

where $L_{(t)}$ = predicted length at time t;
L_{∞} = mean asymptotic fork length;
k = the growth coefficient (yr^{-1}); and
t_o = theoretical age at length=0

In addition to the 3-parameter VBGF model (detailed above), a modified 2-parameter model (von Bertalanffy, 1938) was also fitted that incorporates a set, more biologically meaningful size at birth intercept (L_0) rather than t_0. For the 2-parameter model, L_0 was set to 25 cm STL per estimates from Nammack et al. (1985), Campana et al. (2009) and Bubley et al. (2012).

Finally, a Gompertz growth function was also applied, expressed as described in Ricker (1975):

$$L_{(t)} = L_0\, e^{G(1-e(-kt))} \qquad (3)$$

where G is the instantaneous rate of growth at time t, and k determines the rate of decrease of G; all other parameters are defined as specified for the VBGF.

Parameter estimates for each growth function were estimated using nonlinear least-squares regression methods in R (R Development Core Team 2021). Final model selection was based on known biological parameters and model goodness-of-fit, which was evaluated by the bias-corrected form of the Akaike information criterion (AIC_c; Burnham and Anderson 2002). The AIC_c difference (Δ_i) of each model was calculated based on the lowest observed AIC_c. Models with values of $\Delta_i < 2$ were considered to have strong support. Ninety-five percent confidence intervals were constructed for parameter estimates via bootstrap methods using the "nlstools" package in R (Baty and Delignette-Muller 2011). Nested models were constructed to assess differences in all parameter estimates between sexes, and subsequently sex-specific models were constructed for comparison.

Results

A total of 1,616 spines were assigned an initial age based on growth band counts (males=571, females=1,045; Figure 1). Double band counts were accomplished for 424 spines (26.2%), and double spine diameter measurements were accomplished for 193 spines (11.9%). For double band counts, percent agreement (PA) for ages was 35.6%, Average Percent Error (APE) was 23.4% and coefficient of variation (CV) was 33.1%, with significant negative bias between first and second reads at several age classes (Figure 2, Table 1). Ageing error estimates were substantially higher than published rates from other dogfish age studies (e.g., 8.0% - 12.5% CV; Bubley et al. 2012, Campana et al. 2006, 2009) and higher than typically deemed "acceptable" for informing growth models and assessments (Campana 2001). Conversely, spine measurements were precise, with few measurements falling outside the 95% confidence intervals and no discernable bias pattern in measurements (Figure 3).

The majority of the double counts referenced above (n=231) were generated due to perceived problems with initial estimates. Upon review of preliminary length-at-age data, it was apparent that the age estimates for young dogfish 0-2+ years old did not exhibit the growth trajectory expected for early life, which is typically rapid and nearly linear over the first few years; instead, length-at-age in unworn spines for these years was essentially flat (Figure 4A). Upon re-examination, it was determined that annuli counts for early age classes were compromised due mostly to ager inexperience with enumerating bands in young dogfish spines. Hence, a second set of counts were generated for all fish initially aged 0-4 years, and for two additional fish initially aged 5 and 6 years, respectively. These new ages (Figure 4B) were significantly different from their initial estimates as evidenced by bias comparisons between the two reads for these age classes along (Figure 5) with year classes 1-3 significantly over-aged initially. Second reads from older age classes also showed bias toward initial over-ageing, but high variability and low sample size precluded additional insights. While updated length-at-age using these second reads is more biologically plausible, the flat growth trajectory is apparent throughout the majority of other age classes of unworn spines (Figure 4B). Combined with stark under-ageing of some age 5 and 6 fish, it is likely that ageing error persists throughout the sample set, yielding high uncertainty and low confidence for all age estimates herein.

We nonetheless assessed the potential for using these updated ages to inform growth models for assessment. In addition to using the second, more plausible reads for ages 0-6 where available, we excluded an additional 93 spines from further analysis that had double counts differing by more than 2 years. Second reads were also used in calculating corrected ages for worn spines per *Equation (1)*.

The revised sample set (n=1339) consisted of 901 females and 438 males for generation of growth model estimates. Sex-specific growth models produced the best fits, and AIC values for all models suggest they fit relatively closely to each other (Table 2). However, visual inspection of all growth curves shows poor fit to length-at-age data, particularly in the 0-6 year age classes (Figures 6-7). The 3 parameter VBGF produced the lowest AIC values for females and equivalent

AIC to the 2 parameter VBGF in males, hence it was considered the best model for both sexes. In comparison with previously published growth parameter estimates (Table 3), models from this study suggest a smaller maximum size for both sexes, with slower growth for both males and females relative to the Nammack model.

Maximum observed ages in the current study were older than those of previous studies by 11 years for males and 8 years for females, but it is notable that the current sample set included only 6 individuals > 100cm STL, which appears to be substantially fewer than were included in the Nammack et al. (1985) study and others. Minimum sizes sampled in this study are comparable to those in Nammack et al. (1985) and other studies, but minimum spine base diameter for the current study (1.01 mm) was smaller than in any other published study, with n=58 spines with base diameter less than the minimum size of ~1.5 mm from Nammack et al. (1985) and Ketchen (1975). The presence of these smaller spines in our sample set may indicate changes to morphometric relationships or size-at-birth in the sampled population relative to those from previous studies.

Conclusions

Despite best efforts, the ages generated for the current study are unreliable based on the extremely high error metrics and significant bias between initial and second reads. Without double reads on each spine, it is impossible to verify the validity of ages, and time and resources were not available to pursue double reads for all spines. This is a common issue with production ageing for stock assessment. Results from this study suggest maximum length and age range may be truncated in the current US Atlantic population of *S. acanthias* relative to those documented in previous studies, especially in mature females. However, further work is needed to establish whether larger individuals than were sampled for this study are present within the population and simply not captured in sampling, or are truly absent from the population. Further, more work is needed to clarify ageing criteria for younger fish aged 0-5, as well as to document current size at birth and spine morphometrics across a broader age range in order to create an accurate worn spine correction model specific to this population.

Research recommendations

1) Establish a routine collection program of spines from the entire size and geographic range of *S. acanthias*, including fish from different fishery-dependent and -independent sources, in an effort to accurately characterize the population size and age structure. Collaborators who have worked on Atlantic spiny dogfish age should be sought out to develop best practices and more inclusive sample sets for the next iteration of this study, and sufficient time and resources (i.e. funding) should be devoted to this purpose.
2) Targeted sampling of near-term embryos and neonates is needed to assess timing, identification criteria, and spine base diameter at first annulus deposition to better inform ageing of young fish.

3) Better routine cleaning protocols for spines immediately upon collection to reduce the need to remove dried-on tissue, which risks damage to spine enamel.

Figure 1. Size class groupings by sex for all *Squalus acanthias* aged for this study (n=1616).

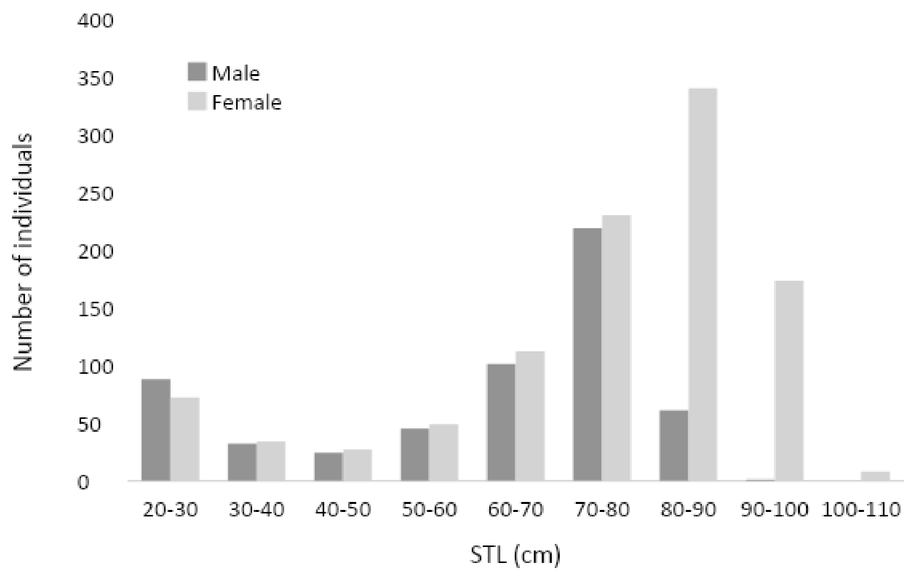

Figure 2. Boxplot of *Squalus acanthias* length-at-age of unworn spines using (A) original ages (n=318) and (B) updated ages (n=302) as determined from raw growth band counts. Thick black lines represent median length at age, shaded boxes are 1st and 3rd quartiles, and bars represent minima and maxima exclusive of outliers (open circles).

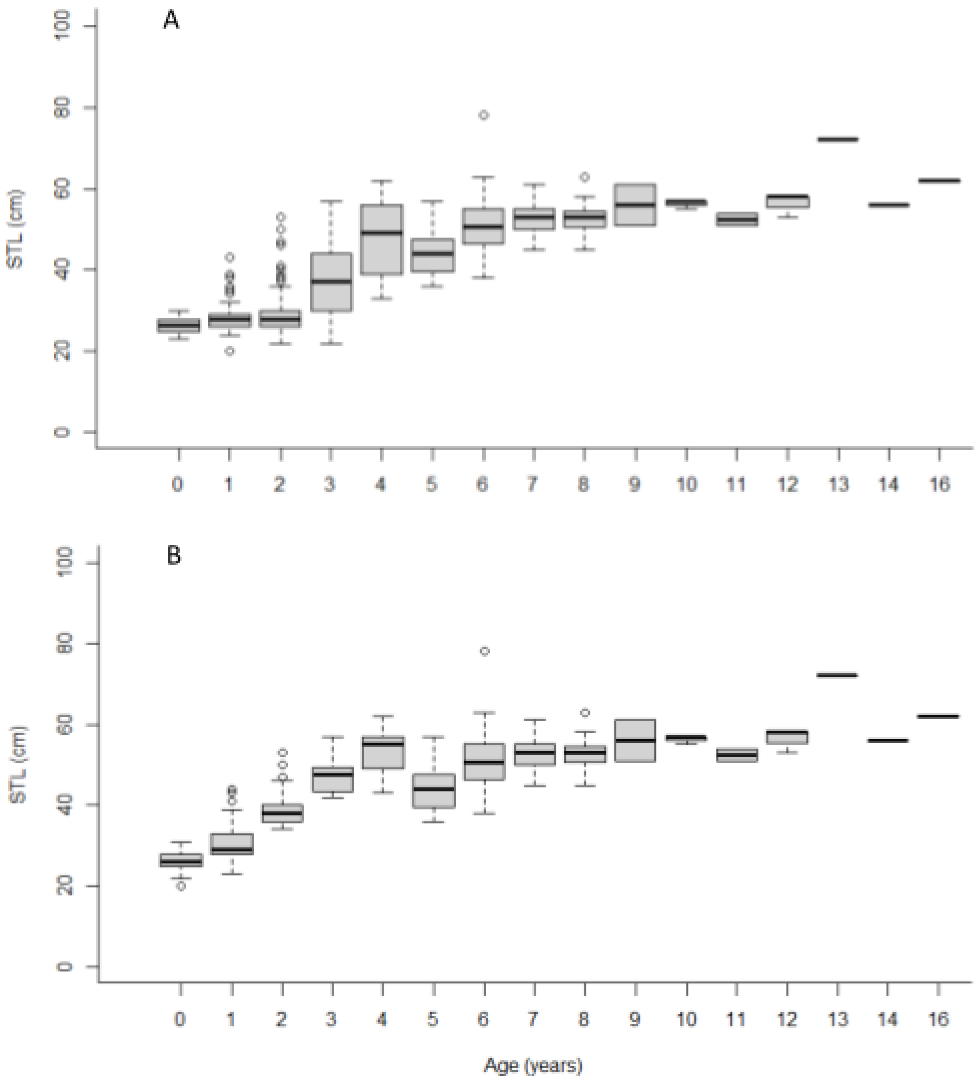

Figure 3. Mean (points), range (black intervals), and confidence intervals (grey intervals) of differences in *Squalus acanthias* spine age estimates between two reads at the estimates for the first read. Open points represent mean differences in age estimates that are significantly different from zero (dashed gray horizontal line). Marginal histograms are for age estimates of the first read (top) and differences in age estimates between reads (right).

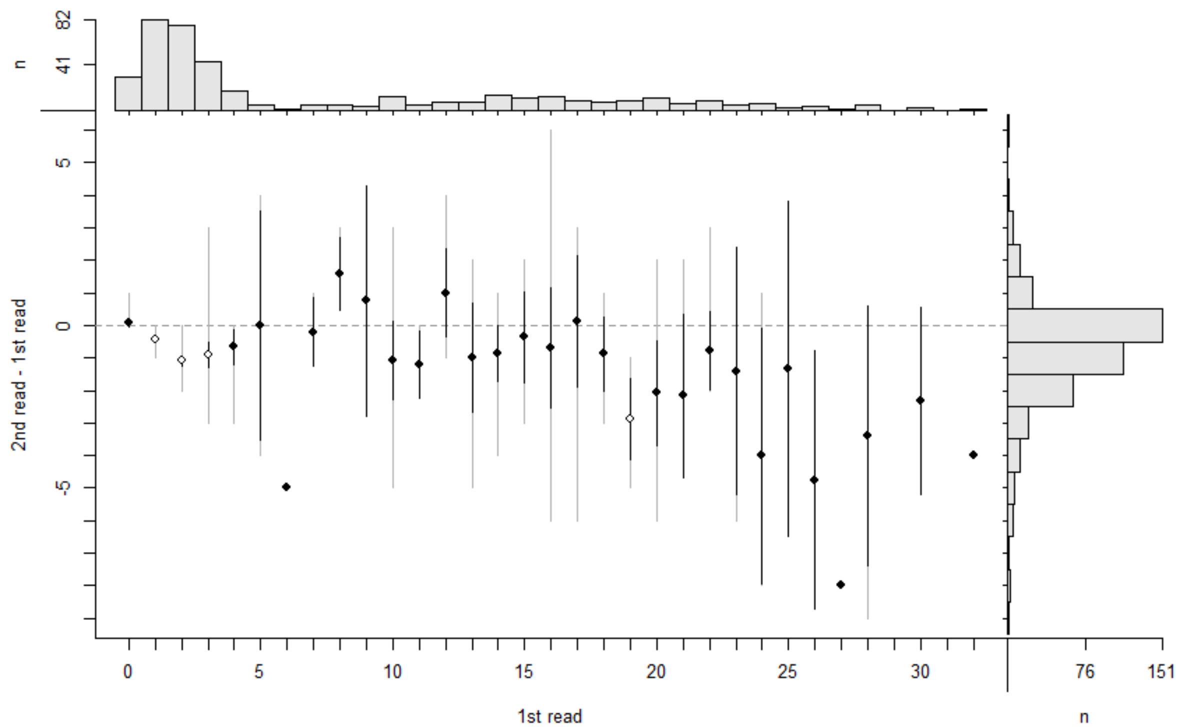

Figure 4. Bland-Altman plot of repeated *Squalus acanthias* spine base measurements (n=193). Black line denotes the mean difference (in mm) between double measurements relative to base diameter (mm), and red dashed lines represent 95% confidence intervals for measurements.

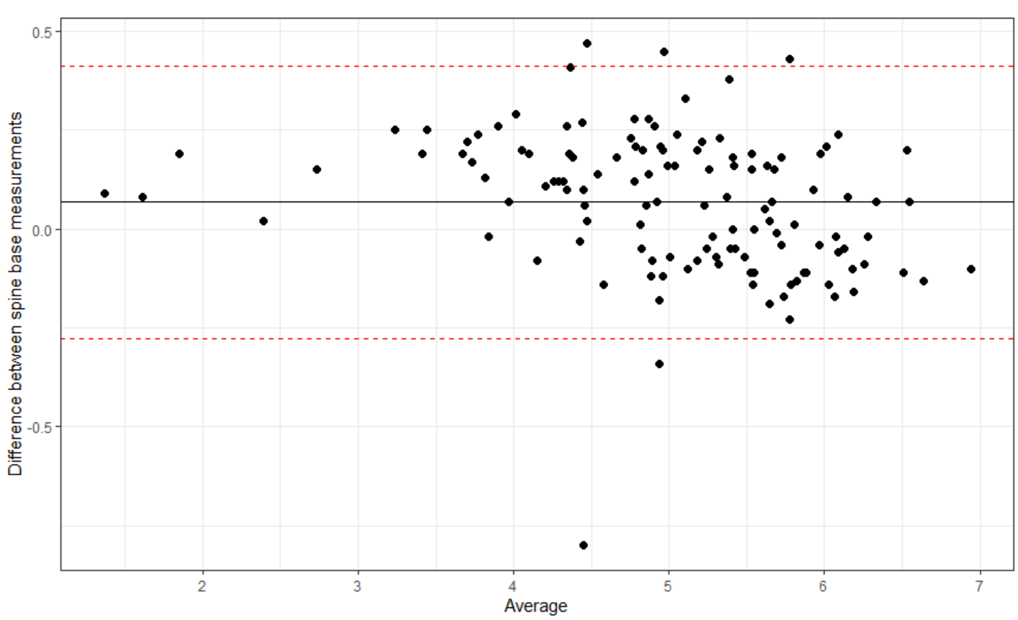

Figure 5. Bias plot of double reads for *Squalus acanthias* ranging in age from 0-6 years. Mean (points), range (black intervals), and confidence intervals (grey intervals) are denoted. Open points represent mean differences in age estimates that are significantly different from zero (dashed gray horizontal line). Marginal histograms are for age estimates of the first read (top) and differences in age estimates between reads (right).

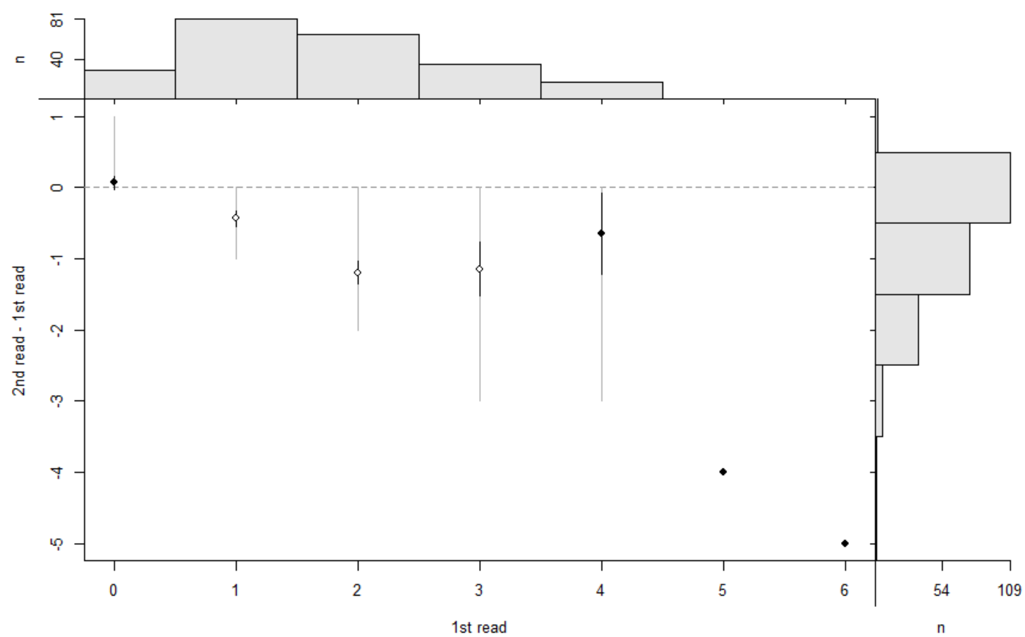

Figure 6. Boxplot of female *S. acanthias* length at age. Growth curves are plotted from growth models fitted in this study as well as the Nammack et al. (1985) growth model, for comparison. Thick black lines represent median length at age, shaded boxes are 1st and 3rd quartiles, and bars represent minima and maxima exclusive of outliers (open circles).

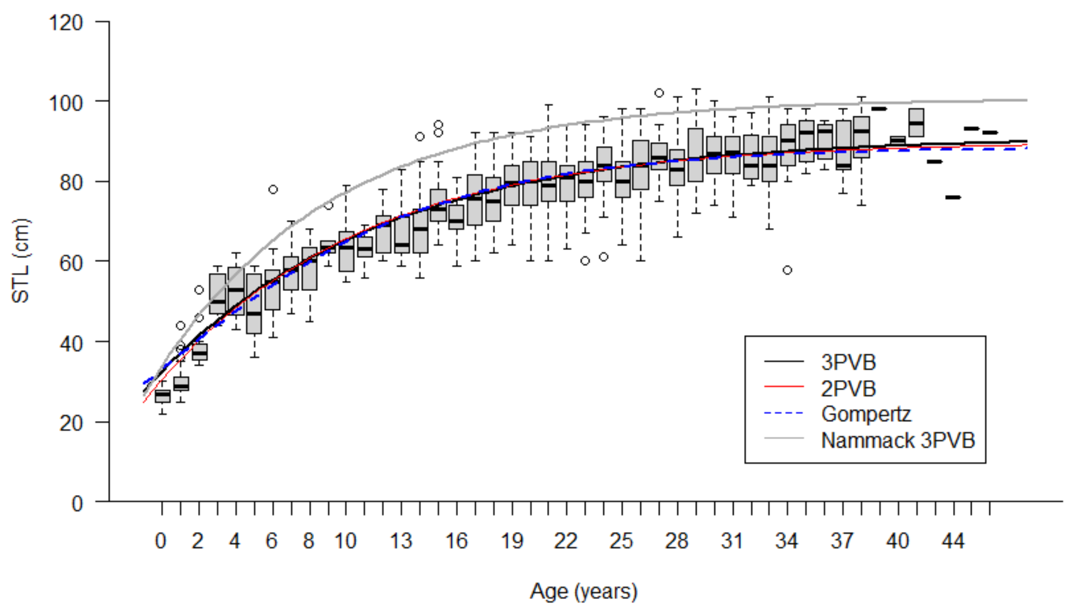

Figure 7. Boxplot of male *S. acanthias* length at age. Growth curves are plotted from growth models fitted in this study as well as the Nammack et al. (1985) growth model, for comparison. Thick black lines represent median length at age, shaded boxes are 1st and 3rd quartiles, and bars represent minima and maxima exclusive of outliers (open circles).

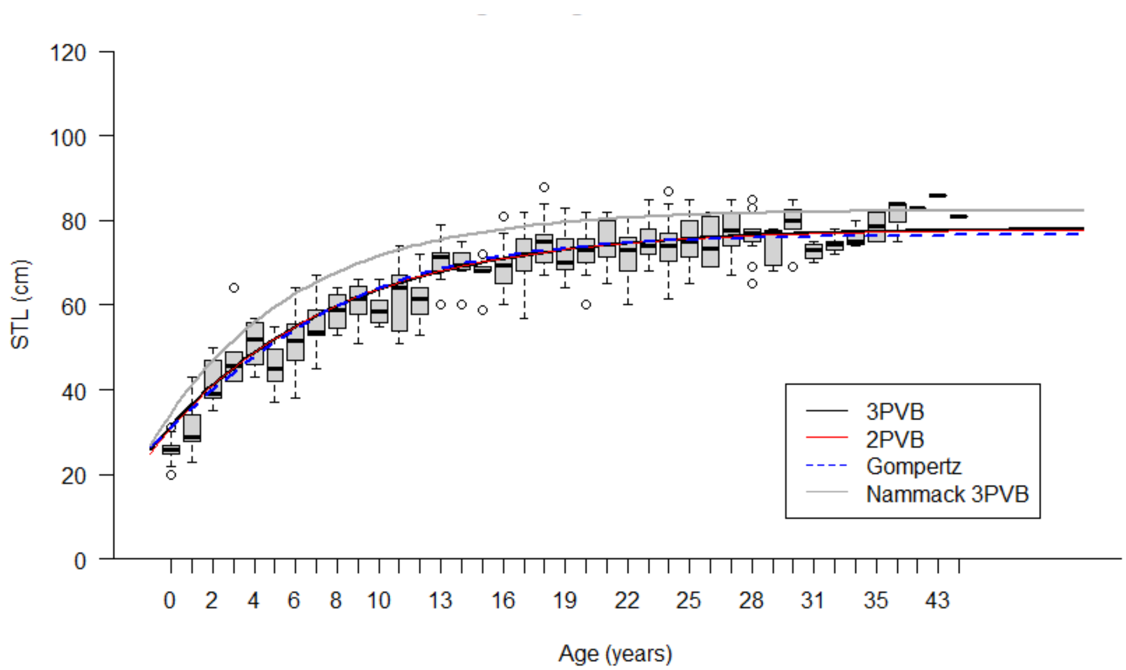

Table 1. Ageing error matrix for all double-read *Squalus acanthias* spines in this study.

Age (1st read)	n	Min	Max	Mean	SE	t	p	LCI	UCI
0	30	0	1	0.07	0.05	1.44	1.0000	-0.03	0.16
1	82	0	1	0.57	0.05	-7.77	0.0000	0.46	0.68
2	77	0	2	0.91	0.08	-13.84	0.0000	0.75	1.07
3	44	0	6	2.09	0.19	-4.81	0.0005	1.71	2.47
4	18	1	4	3.33	0.26	-2.61	0.4045	2.79	3.87
5	5	1	9	5.00	1.26	0.00	1.0000	1.49	8.51
6	1	1	1	1.00					
7	5	6	8	6.80	0.37	-0.53	1.0000	5.76	7.84
8	5	9	11	9.60	0.40	4.00	0.3844	8.49	10.71
9	4	7	12	9.75	1.11	0.68	1.0000	6.22	13.28
10	13	5	13	8.92	0.55	-1.96	1.0000	7.73	10.12
11	5	9	11	9.80	0.37	-3.21	0.6721	8.76	10.84
12	8	11	16	13.00	0.57	1.76	1.0000	11.66	14.34
13	8	8	15	12.00	0.71	-1.41	1.0000	10.33	13.67
14	14	10	15	13.14	0.39	-2.20	0.8891	12.30	13.99
15	11	12	17	14.64	0.62	-0.58	1.0000	13.25	16.02
16	13	10	22	15.31	0.84	-0.82	1.0000	13.47	17.14
17	9	11	20	17.11	0.87	0.13	1.0000	15.10	19.12
18	8	15	19	17.13	0.48	-1.82	1.0000	15.99	18.26
19	9	14	18	16.11	0.54	-5.36	0.0169	14.87	17.35
20	12	14	22	17.92	0.73	-2.84	0.3844	16.30	19.53
21	6	17	23	18.83	0.98	-2.21	1.0000	16.31	21.35
22	9	20	25	21.22	0.52	-1.49	1.0000	20.02	22.42
23	5	17	24	21.60	1.36	-1.03	1.0000	17.81	25.39
24	6	16	25	20.00	1.53	-2.62	0.8891	16.07	23.93
25	3	22	26	23.67	1.20	-1.11	1.0000	18.50	28.84
26	4	18	24	21.25	1.25	-3.80	0.6721	17.27	25.23
27	1	19	19	19.00					
28	5	19	27	24.60	1.44	-2.37	1.0000	20.62	28.58
30	3	27	29	27.67	0.67	-3.50	1.0000	24.80	30.54

Table 2. Comparison of growth models fitted to spiny dogfish length-age data for ages 0-46 (males) and 0-48 (females). Parameters are given ± standard error, except for calculated or fixed parameters (L_∞ for Gompertz models, fixed L_0 for VB 2 parameter models). Akaike Information Criteria (AIC) was used to determine best fit, and models with ΔAIC<2 were considered to have equivalent fit. VB=von Bertalanffy, L_∞ = theoretical maximum length (cm), k = growth coefficient, L_0 = size at birth.

Sex	Growth Function	L_∞	k	L_0	AIC	ΔAIC
Separate	Sex-specific VB, 3 parameter	--	--	--	10043	0.00
Separate	Sex-specific VB, 2 parameter	--	--	--	10052	9.00
Separate	Sex-specific Gompertz	--	--	--	10078	35.0
Combined	VB 3 parameters combined	88.3 ± 0.676	0.085 ± 0.003	27.1 ± 0.611	10283	240
Combined	VB 2 parameters combined	87.3 ± 0.592	0.091 ± 0.003	25	10295	252
Combined	Gompertz combined	85.5	0.121 ± 0.004	28.5 ± 0.506	10329	286
Female	VB 3 parameters	91.0 ± 0.871	0.083 ± 0.004	27.6 ± 0.788	6671.1	0.00
Female	VB 2 parameters	89.9 ± 0.751	0.090 ± 0.003	25	6679.4	8.25
Female	Gompertz	88.4	0.115 ± 0.004	29.4 ± 0.732	6696.0	24.9
Male	VB 2 parameters	78.0 ± 0.570	0.120 ± 0.005	25	3307.3	0.00
Male	VB 3 parameters	78.3 ± 0.616	0.117 ± 0.005	25.7 ± 0.564	3307.7	0.37
Male	Gompertz	76.6	0.161 ± 0.006	26.6 ± 0.533	3315.9	8.55

Table 3. Preferred growth model parameters for Atlantic *S. acanthias* from the current study and previously published studies for comparison.

Study	Sex	Growth Function	L_∞	k	L_0	Max observed age	Max length
Nammack et al. (1985)	M	VB 3 parameter	82.5	0.148	26.9	35	90
	F	VB 3 parameter	100.5	0.107	26.6	40	110
Campana et al. (2009)	M	VB 2 parameter	78.0	0.099	25 (f)	31	85
	F	VB 2 parameter	119.5	0.042	25 (f)	29	100
Bubley et al. (2012)	M	VB 2 parameter	91.5	0.106	25 (f)	22	84
	F	VB 2 parameter	107.2	0.081	25 (f)	28	102
This study	M	VB 3 parameter	78.3	0.117	25.7	46	88
	F	VB 3 parameter	91.0	0.083	27.6	48	103

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