Age Validation from Tagged School and Gummy Sharks Injected with Oxytetracycline

Terence I. Walker, Lauren P. Brown, and John G. Clement

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97/110 Age Validation from Tagged School and Gummy Sharks Injected with Oxytetracycline

Principal Investigator

Terence I. Walker
Marine and Freshwater Resources Institute
PO Box l 14 Queenscliff VIC 3225
Telephone: (03) 5258 0lll, Fax: (03) 5258 0270
Email: Terry.Walker@nre.vic.gov.au

Collaborative Investigator

Professor John G. Clement
Head of Oral Anatomy, Medicine and Surgery
The School of Dental Science
The University of Melbourne
Parkville VIC 3052
Telephone: (03) 9344 6099, Fax: (03) 9347 6739
Email: j.clement@dent.unimelb.edu.au

Objectives

Further refine age validation of sharks by laboratory processing of vertebrae from recaptured tagged school sharks and gummy sharks injected with oxytetracycline as part of the recently completed FRDC funded shark tagging and nursery projects.

Non-technical summary

The present study makes use of vertebrae collected from sharks tagged, injected with oxytetracycline, and released into the wild as part of four successfully completed FRDC projects undertaken during the 1990s. The study successfully met the objective of further refining age validation of methods adopted for routine ageing of gummy sharks and school sharks in the laboratory.

The study was designed to follow up on the conclusion from one of the earlier projects that hypermineralized growth-increment bands are formed annually during winter, and designed to test the assumption of annual periodicity of the bands made when ageing sharks. The study was also designed to test whether the assumption holds for a range of sizes of gummy shark and school shark. The tests were made for growth-increment bands elucidated by the Alizarin Staining Method and the major bands elucidated by the Microradiographic Method. This is achieved by comparing the number of growth-increment bands formed in a vertebra outside the oxytetracycline mark viewed by the application of fluorescence microscopy with the number of winters a recaptured tagged shark was at liberty.

The present report provides the results of analyses of oxytetracycline-marked vertebrae from tagged sharks collected and processed in the laboratory by the end of the year 2000. Of the 253 oxytetracycline-marked vertebra samples embedded in dental compression compound and sectioned, only those taken from sharks at liberty for >1

year (94 sharks) were further processed in the laboratory. Of these, the vertebrae of up to 68 gummy sharks and 18 school sharks could be read to provide measurements and counts of the number of growth-increment bands elucidated by either the Alizarin Staining Method or the Microradiographic Method. The Alizarin Staining Method involved measuring and electronically recording the positions of growth-increment bands and the vertebra margin on the face of the articular cup of a stained vertebra-half. The Microradiographic Method involved measuring and electronically recording the positions of growth-increment bands and the vertebra margin on a microradiograph of a thin section (100 μm thick) removed by longitudinally cutting the vertebrae. Fluorescence microscopy was used for measuring and electronically recording the positions of the fluorescent oxytetracycline mark and the vertebra margin on the thin section.

The Alizarin Staining Method and the Microradiographic Method give very similar results and the predicted mean number of growth-increment bands per winter determined for both methods is very close to 1 for gummy shark and reasonably close to 1 for school shark. For gummy shark, the band counts are slightly higher for females than for males, but the effect of length of shark does not affect the count. For school shark, the study indicates that both methods underestimate the age of sharks ≥1400 mm total length; this compares with ≥1300 mm total length indicated by an earlier study. The results for school sharks are based on oxytetracycline-marked vertebrae from only 18 recaptured tagged animals. Hence, there is a need to continue to encourage the return post-cranial vertebrae of recaptured tagged sharks by fishers, to periodically process these vertebrae in the laboratory, and to periodically update and report the results of statistical analysis of the data.

For both gummy shark and school shark, most available vertebra samples were collected from sharks at liberty for 1–2 years after being tagged and released; only a small number were collected from sharks at liberty 3–5 years. Any bias or lack of precision in counting growth-increment bands is more significant for sharks at liberty for short periods than they are for sharks at liberty for long periods. Hence, data from animals at liberty for long periods are the most valuable for age validation purposes.

It is expected that tagged animals with oxytetracycline-marked vertebrae will continue to be returned over the next 10 years for gummy shark and next 40 years for school shark. Although the number of vertebra samples continuing to be collected is low, those that are returned will be particularly valuable because of their long periods at liberty. These will be processed from time to time in the future for updating the analyses. Arrangements have been made to undertake this work through the ongoing Southern Shark Monitoring Project funded by the Australian Fisheries Management Authority.

The results support the currently adopted cost-effective Alizarin Staining Method for routine ageing of gummy sharks of all lengths and ageing of school sharks of total length <1400 mm. The present study demonstrates that there are no advantages of changing to the more costly Microradiographic Method for routine ageing.

Key words

Shark vertebra age validation, alizarin stain, microradiography, fishery monitoring.

Acknowledgments

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The project has benefited from the cooperation and support provided by Dennis Rowler and David Thomas of the School of Dental Science at The University of Melbourne. The school provided access to and training in the use of specialised equipment to embed and section oxytetracycline marked shark vertebrae and provision of equipment for microradiography, fluorescence microscopy, and electronic storage of fluorescent images.

Several people at the Marine and Freshwater Resources Institute are also acknowledged. Simon Robertson and Corey Green of the Central Ageing Facility provided training and advice, and access to their customised image analysis system Optimate™. Anne Gason provided statistical advice and application of the statistical package SAS.

The Fisheries Research and Development Corporation (FRDC) is acknowledged for funding the project and providing positive feedback through the reporting process. Alex Wells formerly of FRDC is thanked for comments on the draft final report.

FINAL REPORT

97/110 Age Validation from Tagged School and Gummy Sharks Injected with Oxytetracycline

Background

Scope of report

The project 'Age Validation from Tagged School and Gummy Sharks Injected with Oxytetracycline' (FRDC Project 97/110) follows on from four successfully completed projects. These are the 'Southern Shark Age Validation' (FRDC Project 91/037), 'Southern Shark Nursery' (FRDC 93/061), 'Southern Shark Tagging' (FRDC Project 93/066), and 'Southern Shark Tag Database' (FRDC Project 96/162). The present project makes use of vertebrae collected from sharks tagged, injected with oxytetracycline, and released into the wild as part of these projects. These vertebrae were collected for the purpose of further age validation.

The present report provides the results of analysis of samples of oxytetracycline marked vertebrae collected and processed in the laboratory by the end of the year 2000. Most available vertebra samples were collected from sharks at liberty for 1–2 years after being tagged and released; only a small number were collected from sharks at liberty 3–5 years. The longer an animal is at liberty, the more valuable it is for age validation purposes. This is because bias and imprecision in counting growth-increment bands are more significant for sharks at liberty for short periods than for those at liberty for long periods. Although the number of vertebra samples continuing to be collected is low, those that are returned in the future will be particularly valuable because of their long periods at liberty. These will be processed from time to time in the future for updating the analyses.

Previous research

Knowledge of the age and growth of sharks forms an integral component of the stock assessments of gummy shark (Walker 1992; Walker 1994a; Walker 1994b; Walker 1998) and school shark (Punt *et al.* 2000; Punt and Walker 1998). Studies of age and growth have been undertaken on gummy shark (Moulton *et al.* 1992; Walker 1983) and school shark (Moulton *et al.* 1992). In addition, vertebrae are routinely collected from commercially landed sharks as part of the ongoing AFMA funded Southern Shark Monitoring Project, of which many, but not all, have been processed for age determination (Walker *et al.* 2001a). The method of shark ageing adopted for all these studies involves counting growth-increment bands on the stained face of the articular

cup of the centrum of whole vertebrae. This is referred to as the Alizarin Staining Method.

There is a need to test the assumption of annual periodicity for the formation of the growth-increment bands. Testing the assumption requires a process of 'verification' and 'validation'. Verification is the process of evaluation of the assumption and 'age verification' is the process of confirming an age estimate by comparison with age estimates from other indeterminate methods. The process of 'age validation' is the conclusion that the bands counted are deposited predictably, after testing hypotheses about the temporal periodicity of band deposition. This requires substantiating the accuracy of an age estimate by comparison with a determinate method, and the process must be undertaken for all age classes available (Cailliet and Radtke 1987; Cailliet *et al.* 1986; Cailliet and Tanaka 1990).

The assumption of annual periodicity of growth-increment bands was partly verified by demonstration that von Bertalanffy growth curves produced from length-at-age data are similar to those produced from tag length-increment data. However, it was also concluded that ages of school shark longer than 1300 mm total length (estimated at a mean age of 11 years) were underestimated. This is because there are difficulties in resolving growth-increment bands at the vertebra margin of large sharks (Moulton *et al.* 1992).

Further validation of the ageing method was undertaken as part of the 'Southern Shark Age Validation Project' (FRDC Project 91/037) (Walker *et al.* 1995). The project contributed to verifying the assumption of annual periodicity. This was achieved by demonstrating that the number of growth-increment bands counted by the Alizarin Staining Method are consistent with the number of major hypermineralized increments counted on microradiographs by what is referred to as the Microradiographic Method. In addition to providing a comparison of counts from the Alizarin Staining Method and the Microradiographic Method, the project provided an evaluation of readability of vertebrae, within-reader bias and precision, and between-reader bias and precision for counting growth-increment bands. It also provided a comparison of counts for vertebrae from the post-cranial, dorsal fin, and caudal regions of the vertebral column (Officer *et al.* 1996).

The project also provided valuable insights into the structure (Officer 1995), three-dimensional growth (Clement *et al.* 1992), appositional growth (Officer 1995), and heterogeneity in growth (Troynikov and Walker 1999) of shark vertebrae. The project also provided strong evidence for the hypothesis for 'the phenomenon of apparent change of growth rate of gummy shark caused by length-selective fishing mortality' (Walker *et al.* 1998). Attempts were made, as part of that project, to validate the assumption of annual periodicity using sharks held captive in a tank of 27 000 litres of water. The project demonstrated that the bands tend to be deposited during the winter months. However, it was concluded that extra growth-increment bands, described as 'disturbance check marks', form in captive sharks because of trauma from initial capture of the shark from the wild and unavoidable handling in captivity (Officer *et al.* 1997).

The temporal periodicity of the growth-increment bands for all ages could not be fully validated due to the short holding periods for the captive sharks (<1.5 years) and the

small sample size. Vertebra position along the column affects growth-increment count and larger vertebrae produce higher growth-increment band counts than smaller vertebrae. The difference in count could have resulted from either poor resolution at the margin of small vertebrae or a difference in growth-increment formation between the vertebra positions (Officer *et al.* 1996).

The present age-validation project, using tagged sharks injected with oxytetracycline, was designed to follow up on two particular conclusions from the earlier age validation project.

1. Growth-increment bands of high mineral density stain with alizarin red on whole vertebrae and produce radio-opaque bands in microradiographs of sectioned vertebrae. These bands tend to be deposited during the winter months when overall growth of the sharks is slowest.

2. Age estimates made from counting growth-increment bands in whole vertebrae stained with alizarin red are similar to those made from counting major growth-increment bands in microradiographs of sectioned vertebrae. Whereas there is no bias between these two methods, the latter provides marginally better precision.

Need

The shark fishery of southern Australia is based on gummy shark, school shark, and several other species of temperate-water sharks inhabiting the continental shelf and slope. The catch was 2395 tonnes, carcass weight, valued at more than $14.8 million at the point of first sale in Victoria, Tasmania and South Australia during 2000 (Walker *et al.* 2001b). Most of the catch is consumed in Victoria.

Length-at-age data are an important input to the age-structured fishery stock assessment models adopted by SharkFAG for advice to SharkMAC. Gummy sharks and school sharks are currently aged by counting growth-increment bands on the faces of the articular cups of vertebrae stained with alizarin. Before the present study, the method was only partly validated from experiments on captive sharks and required further validation using sharks from their natural environment. There has been a need to further validate the assumption adopted when undertaking age determinations that counted bands of alizarin red stain are deposited annually.

Objectives

Further refine age validation of sharks by laboratory processing of vertebrae from recaptured tagged school and gummy sharks injected with oxytetracycline as part of the recently completed FRDC funded shark tagging and nursery projects.

Methods

Sample Collection

By the end of 2000, 698 vertebra samples had been collected from 1598 recaptured-tagged gummy sharks and 541 recaptured-tagged school sharks (Brown *et al.* 2000). About a third of the vertebra samples (211 gummy sharks and 42 school sharks, i.e. a total of 253 sharks) were taken from tagged sharks injected with the hard tissue marker oxytetracycline. About 15% of the samples were collected as whole vertebral columns; the other 85% of samples were several vertebrae taken from the post-cranial region of the vertebral column. After collection, the samples were stored at –21°C until required for laboratory preparation. Of the oxytetracycline marked vertebra samples, 37% (94 sharks) were taken from sharks at liberty for >1 year (Figure 1). Of these, 11 sharks were at liberty for >4 years (5 male gummy sharks up to 5.1 years and 6 female school sharks up to 4.6 years).

Laboratory Preparation

All 253 oxytetracycline-marked samples of vertebrae stored frozen were prepared for microradiography, alizarin red staining, and fluorescence microscopy. The samples of vertebrae were thawed and a single vertebra was carefully excised from the post-cranial region and cleaned of connective tissue, ensuring that the outer margins of the vertebral articular cup faces were not damaged. Each excised vertebra and its associated vertebra sample was then stored in 70% ethanol and placed in the dark to minimise loss of fluorescence of the oxytetracycline marker.

In preparation for sectioning, each of the 253 vertebrae was attached to a chuck with dental-impression compound (Type 1, Kerr Sybron Corporation, California, USA). Each vertebra was sectioned twice longitudinally through the lateral intermedialia using a Lietz 1600 radial saw with a water-cooled, diamond-impregnated metal disc to remove a section 100 μm thick cut to include the centre of the vertebra (hereafter referred to as 'focus'). The larger of the two remaining parts cut from the vertebra (hereafter referred to as a 'vertebra-half') was used for application of the Alizarin Staining Method. The 100 μm thick section was used for application of the Microradiographic Method and for measuring the position of the oxytetracycline mark observed by fluorescence microscopy. Of the 253 oxytetracycline-marked vertebra samples embedded and sectioned, only those taken from sharks at liberty for >1 year (94 sharks) were further processed.

For the Alizarin Staining Method, procedures for preparing and staining the vertebra-halves to improve definition of the concentric growth-increment bands visible on the two faces of the articular cups required several steps. These procedural steps were similar to those adopted in earlier studies of gummy shark and school shark (Moulton *et al.* 1992; Officer *et al.* 1996; Walker 1983; Walker *et al.* 1998). The vertebra-halves were prepared for staining by first removing all connective and facia material with 2% sodium hypochlorite (White King bleach, Household and Boat Care, Victoria). Next, the vertebra-halves were rinsed thoroughly with water and air dried. For reading, each vertebra-half was then immersed for 1–5 minutes in a freshly prepared solution of concentrated Alizarin red stain, buffered with 0.1% potassium hydroxide, to stain for

the growth-increment bands. When stained, the vertebra-half was washed in tap water and the vertebral bands were examined and measured immediately.

For the Microradiographic Method, procedures for producing microradiographs of 100 μm thick sections were similar to those adopted in a previous study of gummy shark and school shark (Officer *et al.* 1996). For initial trials, each 100 μm thick section was placed on a light safe bag containing medium-fine resolution film (AGFA Industrix D2) and radiographed using a Faxitron x-ray machine for 30–60 seconds at 2 mV. However, although these microradiographs were clear enough for counting the growth-increment bands, they were not clear enough for providing accurate measurements of the band positions. Hence, the 100 μm thick sections were subsequently microradiographed using fine resolution film (Kodak SO343) in an OEG tube x-ray machine at 25 kV and 10 mA for 18 minutes. Each microradiograph of the 100 μm thick section was then placed in a labelled slide mount.

Growth-increment band measurement and count

For the Alizarin Staining Method and the Microradiographic Method, each vertebra was processed by the one reader who assigned a 'readability score' of 1–5 based on the degree of differentiation of growth-increment bands and the difficulty in interpreting the arrangement of the bands. The readability scores were defined as follows: (1) bands unambiguous with exceptional clarity, (2) bands unambiguous but of diminished clarity, (3) two band counts possible but the recorded count is the more likely, (4) more than two band counts possible but the recorded count most likely, and (5) no count possible and recorded as 'unreadable' (Officer *et al.* 1996). Before taking any readings, the reader practised identifying and counting bands by calibrating readings against previous readings produced by more experienced readers from an archived age reference set of vertebrae for gummy shark and school shark collected during 1991–93.

On each vertebra, a series of measurements was made for each of four types of measurement using the Bioscan image analysis software Optimas™ and Optimate™. For a series of measurements, the distance of the proximal edge of each growth-increment band and the distal margin of the vertebra from the 'focus' were measured on a 'transect' in millimetres. The positions of the growth-increment bands were measured at magnifications of x10 and x25. The first series of measurements was taken on the 'articular face' of the vertebral articular cup stained vertebra-half (Figure 2a). The second series was taken on the 'sectioned face' of the stained vertebra-half exposed by the longitudinal cut (Figure 2b). The third series was taken on the microradiograph of the 100 μm thick section (Figure 2c). The fourth series, which included only the oxytetracycline mark and vertebra margin, was taken on the actual 100 μm thick section directly under fluorescence microscopy (Figure2d). These four series of measurements provided a statistical basis for comparing the number and positions of the growth-increment bands observed using the Alizarin Staining Method and with those using the Microradiographic Method. The positions of the growth increment bands from the Alizarin Staining Method and the Microradiographic Method were each compared with the position of the oxytetracycline mark. This way the positions and number of growth-increment bands outside the oxytetracycline mark near the margin of the vertebra could be compared with the number of winters the sampled shark was at liberty, for both methods. For the purpose on testing for reader bias and reader precision, the first and

second series of measurements were repeated three months after taking the initial measurements.

For the Alizarin Staining Method, the growth-increment bands and vertebra margin observed on one of the two 'articular faces' and on the one 'sectioned face' of the stained vertebra-half were measured under reflected light. The first series of measurements on the 'articular face' is designated AS1. For this series of readings, the 'articular face' of the articular cup was orientated such that the longest of the four corpus calcareum arms lay in the horizontal plane (Figure 2a); this provided the greatest separation and hence the greatest resolution of growth-increment bands. A 'transect', placed on the 'articular face' close to the edge of longitudinal cut through the vertebra, was adopted for taking the measurements. In most samples a 'birth band' was observed and measured; the 'birth band' is designated BB. The second series of measurements was required to relate the first series of measurements to the third series of measurements taken on the microradiograph of the 100 μm thick section and to the fourth series of measurements taken on the 100 μm thick section under fluorescence microscopy. The second series of measurements was taken by orienting the stained vertebra-half with the 'sectioned face' of the longitudinal cut through the vertebra to lie in the horizontal plane (Figure 2b).

The second series of measurements, designated AS2 and taken on the 'sectioned face' of the vertebra-half, were approximately contiguous to the series measurements of the growth-increment bands and the vertebra margin on the 100 μm thick section. Hence, the different sets of measurements could be related to each other by the careful positioning of the 'transects'. The 'transect' on the 'section face' was positioned to coincide with the position of the 'transect' placed on the microradiograph and to coincide with the position of the 'transect' placed directly on the 100 μm thick section for measuring the oxytetracycline mark under fluorescence microscopy. For all three of these types of measurement, the 'transects' were from the focus to the vertebra margin on the longest corpus calcareum arm. The second, third, and fourth series of measurements were, in effect, taken approximately in the one plane of the longitudinal section through the vertebra; only the first series of measurements (taken on the 'articular face') were not taken in this plane.

For the Microradiographic Method, the 'major growth-increment bands', designated MR1, and the 'major and minor growth-increment bands combined', designated MR2, were measured under transmitted light on the microradiographs of the 100 μm thick section. The major bands were identified as broad radio-opaque bands observed in the corpus calcareum, often running into the intermedialia and associated with an indentation on the exposed articular face. The minor bands were identified as narrow and less distinct radio-opaque bands (Figure 2c).

Standard fluorescence microscopy equipment was inadequate for producing an electronic image of the fluorescent oxytetracycline mark present on the 100 μm thick section. Hence, it was necessary to use a low light level video camera (SPOT 32) to produce the required image. The position of the fluorescent oxytetracycline mark and the vertebra margin were measured from the focus (Figure 2d); this series of two measurements is designated OM1.

To compare the relative positions of the growth-increments bands determined from the Alizarin Staining Method and from the Microradiographic Method with the relative position of the oxytetracycline mark viewed under fluorescence microscopy it was necessary to standardise the AS1, MR1, MR2, and OM1 series of measurements. These were all standardised against the AS2 measurements based on vertebra margin measurements using several equations. The addition of 0.2 mm in the first equation was to adjust for the curvature of the articular cup.

$$AS1_{stand} = AS1 + (AS2_{margin} - AS1_{margin}) + 0.2 ,$$

$$MR1_{stand} = MR1 \times AS2_{margin} / MR1_{margin} ,$$

$$MR2_{stand} = MR2 \times AS2_{margin} / MR2_{margin} , \text{ and}$$

$$OM1_{stand} = OM1 \times AS2_{margin} / OM1_{margin} .$$

The number of growth-increment bands formed outside the oxytetracycline mark for each of the Alizarin Staining Method and the Microradiographic Method was determined by counting the number of growth-increment bands with a standardised measurement greater than the standardised oxytetracycline mark measurement. These band counts were made for each of the AS1, MR1, and MR2 series of measurements (Figure 3a,b).

The application of these data for testing the ageing assumption of annual periodicity of growth-increment bands is based on an important conclusion of an earlier study. This conclusion is that growth-increment bands of high mineral density stain with alizarin red on whole vertebrae and produce radio-opaque bands in microradiographs of sectioned vertebrae. These bands tend to be deposited during the winter months when overall growth of the sharks is slowest (Officer 1995; Officer *et al.* 1997; Officer *et al.* 1996; Walker *et al.* 1995). In the present study, the number of winters that a tagged shark was at liberty was determined by counting the number of September months encountered during its time free. It is assumed here that formation of the growth-increment band had started and was clearly visible by September.

Statistically testing for reader bias and precision

The ability of the single reader used in the present study to produce growth-increment band counts consistent with counts from previous studies (Moulton *et al.* 1992; Officer *et al.* 1996) was statistically tested for reader bias and reader precision. These tests were undertaken using the growth-increment counts outside the oxytetracycline mark determined from the paired AS1 series of measurements taken three months apart for the Alizarin Staining Method. Reader bias was tested by a pair-wise comparison of the counts using the ANOVA Duncan's Multiple Range Test for balanced pairs as a repeated measures design. Duncan's Multiple Range Test was also used for determining the effect of readability score on reader bias; this tested for different readability scores among the balanced pairs. Reader precision was tested by the Index of Average Percent Error (IAPE) score (Beamish and Fournier 1981); the smaller the IAPE score the greater the precision.

Statistically testing the hypothesis for annual periodicity of band deposition

Hypermineralized growth-increment bands, opaque on microradiographs and stained by alizarin red, have been demonstrated to be deposited during winter (Officer *et al.* 1997). For the hypermineralized bands to form annually, then one band has to be deposited each year. To statistically test whether or not the bands are deposited annually, the null hypothesis is that the mean number of bands deposited per winter is 1. To reject the null hypothesis, a test is needed to show that the mean number of bands deposited per winter is significantly different from 1. The null hypothesis was tested using generalised linear modelling.

The generalised linear modelling was undertaken using the GENMOD procedure, which is part of the statistical package SAS/STAT (Release Version 8.01) (SAS Institute Incorporated). The GENMOD procedure fits a generalised linear model (Nelder and Wedderburn 1972) to data by maximum likelihood estimation of parameters through an iterative fitting process. The GENMOD Type 3 Analysis was adopted because design of the data is non-orthogonal and because the results do not depend on the order in which the model terms are fitted.

The GENMOD procedure automatically fits a sequence of models, beginning with a simple model with only an intercept term, to include one additional explanatory variable in each successive model until all explanatory variables in the specified model are included. These asymptotic tests allow the statistical significance of each additional explanatory variable to be assessed from the χ^2 value divided by the degrees of freedom (scaled Pearson value) produced for each explanatory variable. The basic model adopted for the test was

$$\text{‘bands per winter’} = \text{‘sex’} + \text{‘length-class’} + \text{‘sex x length-class’} + \varepsilon ,$$

where the input data for each shark were ‘bands per winter’, ‘sex’ and ‘length-class’. In the adopted model, the dependent variable was ‘bands per winter’, which is the number of growth-increment bands counted outside the oxytetracycline mark divided by the number of winters the shark was at liberty. The independent variables were the two categorical variables ‘sex’ of shark and ‘length-class’ of shark and the interaction between these two variables ‘sex x length-class’. For any shark, ‘sex’ was ‘male’ or ‘female’ and ‘length-class’ was ‘small’, ‘medium’, or ‘large’. Categorisation of the sharks by length varied depending on species, sex, and number of sharks with oxytetracycline-marked vertebra samples available. Female gummy sharks attain a much larger maximum length than do male gummy sharks, so the selected ranges in total length of shark were different for the two sexes. The selected three length-classes ‘small’, ‘medium’, and ‘large’ were <1000 mm, 1000–1199 mm, and ≥1200 mm, respectively, for female gummy sharks, and <1000 mm, 1000–1099 mm, and ≥1100 mm, respectively, for male gummy sharks. Female and male school sharks attain similar length, and because the sample size for male school sharks was very low, no attempt was made to test for the effect of sex. The three length-classes of <1000 mm, 1000–1399 mm, and ≥1400 mm were adopted for both sexes. Hence, because the two sexes were combined, a simpler model was adopted for school shark:

$$\text{‘bands per winter’} = \text{‘length-class’} + \varepsilon.$$

The ε term denotes an error term where the individual residuals ε_i in 'bands per winter$_i$' are distributed normally with a mean of zero and constant variance. The error-structure was tested for each model using 'distribution = normal' and 'link = identity' in the SAS procedure GENMOD. For each run of the model, two criteria were adopted for acceptance of the 'normal' error-structure. The first criterion was based on whether or not the model converged. If the model did converge, then, the second criterion was based on 'goodness of fit' of the model to the data. Goodness of fit was evaluated from the value of scaled Pearson's χ^2 / degrees of freedom. If this value was close to 1, then the selected error structure was accepted.

Results

Of 94 tagged sharks injected with oxytetracycline, recaptured, reported with vertebrae from the post-cranial region of the vertebral column, and at liberty for >1 year, 88 displayed the oxytetracycline mark under ultra-violet light. The absence of an oxytetracycline mark in 6 sharks might be explained in several ways. The first is the lack of or slow mineralisation of the vertebrae during the first 7–14 days after the oxytetracycline injection. The second is the failure to properly inject oxytetracycline into the body cavity of the shark at the time of tagging. The third is the substitution of the oxytetracycline-marked vertebra sample with a vertebra from a shark not injected with oxytetracycline during handling. To eliminate the possibility of sample substitution during laboratory preparation, a second vertebra sample was taken from long-term storage and tested for the presence of an oxytetracycline mark. This indicated that if sample substitution had occurred, it must have occurred during collection at sea or during initial handling of the sample in the laboratory.

Readability scores of 5 for several samples further reduced sample size for data analysis. For the Alizarin Staining Method, sample sizes were 68 gummy sharks and 18 school sharks for band counts from the AS1 series of measurements. For the Microradiographic Method, sample sizes were 68 gummy sharks and 17 school sharks for band counts from the MR1 series (Table 1). As demonstrated by an earlier study (Officer *et al.* 1996), band counts from the MR2 series were too high to be annual and therefore not used for further analysis.

The ANOVA Duncan's Multiple Range Test grouping indicated that there was no significant difference between the two sets of counts of growth-increment bands outside the oxytetracycline mark determined from the paired AS1 series of measurements taken three months apart for the Alizarin Staining Method. Only data from vertebrae with readability scores 1–4 were included in the test. The IAPE values from the test for precision of the growth-increment count was low for gummy shark (10%) and school shark (13%), suggesting good precision on the part of the reader.

Despite the lack of bias and comparatively good precision on the part of the reader, band count differences of <–2 or >+2 can occur. This is high for the present study where the maximum period is 5.1 years. A variation of even 1 or 2 counts for a period with expected counts 1–5 is high (Table 2).

Generalised linear models were applied to test for the effects of sex and length-class, and their interaction, on the 'number of bands per winter' in gummy shark vertebrae for the Alizarin Staining Method and the Microradiographic Method separately. Similar models were applied to test for the effect of length-class only on the 'number of bands per winter' in school shark (Table 3). When applied all models converged. For gummy shark, the effect of sex was significant ($P<0.01$ for the Alizarin Staining Method and $P<0.05$ for the Microradiographic Method), whereas the effects of length-class and the interaction term 'sex x length-class' were not significant. For school shark, the effect of length-class was highly significant ($P<0.001$ for both methods). The models were applied to also determine the predicted number of bands per winter for gummy shark with the sexes combined and for school shark with the length-classes combined. For gummy shark, the 'scaled Pearson χ^2/df' value (df denotes degrees of freedom) was 1.030 in Model 3, which tested for the effect of sex, and 1.015 in Model 4, which combined the sexes. These values were sufficiently close to 1 to indicate that these two models fit the data acceptably well when assuming a normal probability distribution for the error structure. For school shark, the 'scaled Pearson χ^2/df' value was 1.200 for the Alizarin Staining Method and 1.214 for the Microradiographic Method in Model 5 testing for the effect of length-class. The value was 1.059 for the Alizarin Staining Method and 1.063 for the Microradiographic Method in Model 6, which combines the length-classes. These values for school shark are not as close to 1 as they are for gummy shark, but they are sufficiently close to indicate that these two models fit the data acceptably well assuming a normal probability distribution for the error structure (Table 3).

For each of gummy shark and school shark, the predicted mean number of growth-increment bands per winter, when considered with the standard error (SE) and 95% confidence interval (CI), is sufficiently close to 1 not to reject the null hypothesis that the bands are deposited annually (Table 4). The values for gummy shark are 0.99 (SE 0.07, CI 0.85–1.13) for the Alizarin Staining Method and 1.12 (SE 0.09, CI 0.96–1.29) for the Microradiographic Method. The values for school shark are 0.79 (SE 0.13, CI 0.55–1.04) for the Alizarin Staining Method and 0.89 (SE 0.17, CI 0.54–1.23) for the Microradiographic Method.

For the female and male gummy sharks examined separately, the predicted mean number of bands per winter values suggests that the Alizarin Staining Method of ageing is more reliable for females than for males. The values for females are 1.17 (SE 0.17, CI 0.82–1.51) for the Alizarin Staining Method and 1.27 (SE 0.21, CI 0.85–1.61) for the Microradiographic Method. The values for males are 0.72 (SE 0.11, CI 0.51–0.92) for Alizarin Staining Method and 0.91 (SE 0.13, CI 0.65–1.17) for the Microradiographic Method. The value 1 falls within the range of the confidence interval for the females, whereas the value 1 does not quite fall within the confidence interval for the males for the Alizarin Staining Method, which suggests this ageing method might be underestimating the age of males.

For school sharks in the three length-classes of <1000 mm, 1000–1399 mm, and ≥1400 mm total length examined separately, the predicted mean number of bands per winter values indicate that the ageing method is more reliable for ageing small and medium sized sharks than for large shark. The predicted mean number of bands per winter values for the three length-classes are 1.29 (SE 0.24, CI 0.82–1.75), 0.68 (SE 0.24, CI 0.20–1.15), and 0.25 (SE 0.15, CI 0.00–0.53), respectively, for the Alizarin Staining

Method. These values are 1.60 (SE 0.28, CI 1.04–2.16), 1.05 (SE 0.27, CI 0.51–1.59), and 0.13 (SE 0.16, CI –0.19–0.44), respectively, for the Microradiographic Method.

The results show the same general patterns for both ageing methods, but the Microradiographic Method provided slightly higher band counts than the Alizarin Staining Method (~10%). However, the results provide no basis for determining which of the two methods provides the more reliable age estimates. The results indicate that both ageing methods provide acceptable age estimates for gummy shark, but both methods give highly biased under-estimates of age for school sharks of total length ≥1400 mm.

Discussion

The oxytetracycline marking of gummy sharks and school sharks during recent tagging studies provided the opportunity to further validate the assumption of annual formation of growth-increments bands observed in the vertebrae of these species. The results presented in this report, provide supporting evidence for the annual formation of growth-increment bands stained by alizarin on the face of the articular cup of whole vertebrae, and the major bands identified on microradiographs of longitudinally sectioned vertebrae. The results for gummy shark are clear, but there is a need to process additional school shark vertebrae as they continue to be collected in the future.

There was no significant difference between the two sets of counts of growth-increment bands outside the oxytetracycline mark determined by the one reader used in the present study from the paired AS1 series of measurements taken three months apart for the Alizarin Staining Method. This indicates that the reader exhibited no bias in reading. In addition, the IAPE values from the test for precision of the growth-increment band counts was relatively low for both gummy shark (10%) and school shark (13%). These scores are similar to the IAPE scores for growth-increment counts from the Alizarin Staining Method for an earlier study of gummy shark (8–12%) and school shark (5–11%) involving four separate readers (Officer *et al.* 1996). Given the reader's lack of bias and high precision for Alizarin Staining Method, it is assumed that the reader had similar low bias and high precision for interpretation of major and minor growth-increment bands for the Microradiographic Method.

Generalised linear models testing for the effects of sex and length-class, and their interaction, on the 'number of bands per winter' gave very similar results for the Alizarin Staining Method and the Microradiographic Method in both gummy shark and school shark. This result is consistent with the results of an earlier study that demonstrated that band counts made from the Alizarin Staining Method are similar to counts of major bands made from the Microradiographic Method (Officer *et al.* 1996).

For gummy shark, the predicted mean number of growth-increment bands per winter is very close to 1 (0.99, CI 0.85–1.13) based on vertebrae from 68 animals. However, examining the female and male gummy sharks separately suggests that the mean number of bands per winter determined from the Alizarin Staining Method of ageing might be more reliable for females (1.17, CI 0.82–1.51) than for males (0.72, CI 0.51–0.92). The Microradiographic Method predicted very similar values, but the predicted

mean number of bands for the males was closer to 1 (0.91, CI 0.65–1.17). This suggests that the Alizarin Staining Method might slightly underestimate the age of males. It was important to consider the females and males separately because they have very different growth curves.

For school shark, where the females and males have similar growth curves, the predicted mean number of growth-increment bands per winter is reasonably close to 1 (0.79, CI 0.55–1.04), based on vertebrae from 18 animals. However, the predicted mean number of bands per winter values for each of the three length-classes for school shark separately suggests that the ageing method is more reliable for ageing small and medium sized sharks than large shark. The predicted mean number of bands per winter values for the length-classes <1000 mm (1.29, CI 0.82–1.75) and 1000–1399 mm (0.68, CI 0.20–1.15) are reasonably close to 1. However, the predicted mean number of bands per winter value for the length class ≥1400 mm (0.25, CI 0.00–0.53) is statistically different from 1. Under-estimation of age for large school sharks by the Alizarin Staining Method has been suspected from earlier studies. It was concluded that sharks >1300 mm total length (mean age of ~11 years) cannot be aged reliably because of compaction of growth-increments near the outer edge of the vertebrae of large sharks (Moulton *et al.* 1992). The present study indicates that large school sharks cannot be more reliably aged by the Microradiographic Method; the predicted mean number of bands per winter value for the length class ≥1400 mm is also statistically different from 1 (0.13, CI –0.19–0.44) for this method.

Whereas the present report provides an analysis of the available data, four factors acting together indicate that it would be better to undertake the statistical analysis after oxytetracycline marked sharks have been at liberty for much longer periods. (1) Most of the recaptured-tagged sharks injected with oxytetracycline have been at liberty for only relatively short periods (most <2 years). (2) Imprecision in age estimation from counting growth increment bands is high compared with the short period that the animals with oxytetracycline marked vertebra were at liberty. (3) Deposition of an additional band caused by trauma at the time of injection with oxytetracycline and tag and release. (4) Growth increment bands deposited near the margin of a vertebra (i.e. after deposition of the oxytetracycline mark) are the most difficult to resolve.

For the first factor, most of the recaptured tagged animals with an oxytetracycline mark were recovered after less than one year at liberty, with the numbers declining exponentially in successive years. Animals at liberty for less than one year were excluded from the analysis because many of them would not have had sufficient time to deposit a growth-increment band. Including a large number of these animals would introduce a bias towards underestimating the number of bands per winter, particularly as it takes several months to develop a growth-increment bands of high mineral density, which are radio-opaque and stain with alizarin.

For the second factor, imprecision in age estimation from counting growth increment bands is high relative to the short period at liberty. An earlier study of gummy shark and school shark indicates that a reader can only reproduce the count of alizarin stained bands or of radio-opaque bands on a second round of counting for only ~40% of the counts. Another ~40% of the counts differ by either –1 or +1, and ~20% differ by more than either –2 or +2 or more (Officer *et al.* 1996). The precision of the reader used in the present study was comparable with that of four readers used in the earlier study. In

the present study, all were at liberty for up to only 5.1 years. A variation of 1 or 2 counts for a period with expected counts 1–5 is comparatively high, as indicated by Table 2.

For the third factor, as suggested from an earlier study of gummy shark (Officer *et al.* 1997), another potential source of variation in the count is that some animals might deposit a band resulting from trauma. If this is the case, it is likely it is expected in the present study that initial capture, tagging, and injection of the sharks at the time of tag release might have caused the formation of an extra band or premature formation of a band.

For the fourth factor, bands deposited after deposition of the oxytetracycline mark are the most difficult to resolve, particularly those at the vertebra margin. Earlier studies show that vertebral growth is appositional and occurs at the vertebra margin. A forming or recently formed band is difficult to detect and involves subjective judgement on the part of the reader (Officer 1995).

In relation to four these factors, it follows that the longer a shark is at liberty the more valuable it is for the purpose of these analyses. Vertebrae from tagged sharks continuing to be collected as they are recaptured, so there is the need to periodically update laboratory processing of vertebrae, statistical analysis of the data, and reporting of the results. This is likely to have less effect on the results for gummy shark than on the results for school shark. Data from 68 gummy sharks are presently included in the analyses, and small numbers will continue to be returned over the next 10 years. There may be a marked effect on the results for school sharks because only 18 animals are included in the present analyses. Tagged school sharks injected with oxytetracycline for this species are expected to continue to be returned for the next 40 years.

A fifth factor that might have affected the results is length-selective fishing mortality. An earlier study discovered the ‘phenomenon of apparent change of growth rate’ for gummy shark. This phenomenon is the distorting of growth curves determined from sampling the wild population where the distortion is caused by the effects of gillnets used in the fishery selectively removing large animals from the young age-classes and small animals from the old age-classes in the population (Walker *et al.* 1998). The effect is particularly marked for male gummy sharks in Bass Strait where most of the catch is taken by gillnets of 6-inch mesh-size. Whereas, these effects cannot be readily quantified, tagging of slower-growing animals among the males than among the females may partly explain some of the difference in the mean number of growth-increment bands deposited per winter between the male and female gummy sharks found in the present study.

Benefits

There is a need for confidence in the methods adopted for ageing sharks used for producing length-at-age data for fishery monitoring and stock assessment. The ultimate benefit from improved assessment will contribute to establishing the Southern Shark Fishery as one managed with rehabilitated stocks producing catches at a high sustainable level. This will ensure improved economic viability of the industry for catching and processing sector participants and will contribute to the Australian

economy as a whole. This will ensure an ongoing supply of fresh shark meat so highly esteemed by sections of the Australian community.

The flow of benefits are allocated as 60% Commonwealth, 10% Victoria, 10% Tasmania, 10% South Australia, and 10% Western Australia.

Conclusions

The present study demonstrates that the predicted mean number of growth-increment bands per winter determined by both the Alizarin Staining Method and Microradiographic Method is very close to 1 for gummy shark and reasonably close to 1 for school shark. This supports the assumption made when ageing sharks by these methods that growth-increment bands are annual.

For gummy shark, the band counts are slightly higher for females than males, but the effect of length of shark does not affect the count. For school shark, the study confirms that both methods underestimate the age sharks of length ≥1400 mm total length.

The results for school sharks are based on oxytetracycline-marked vertebrae from only 18 recaptured-tagged animals. Hence, there is a need to continue to encourage the return post-cranial vertebrae of recaptured tagged sharks by fishers, to periodically process these vertebrae in the laboratory, and to periodically update and report the results of statistical analysis of the data. In addition to the school shark, there is benefit in continuing to process gummy shark as the current results are based on 68 animals at liberty 1–5 years. Data from animals at liberty for long periods are the most valuable.

The results support the currently adopted cost-effective Alizarin Staining Method for routine ageing of gummy shark and ageing of school sharks of total length <1400 mm. It cannot be demonstrated from the present study that there are advantages of changing to the more costly Microradiographic Method for routine ageing.

Further development

The longer a shark is at liberty the more valuable it is for the purpose of age validation. It is expected that small numbers of tagged sharks with oxytetracycline marked vertebrae will continue to be returned over the next 10 years for gummy shark and next 40 years for school shark. The vertebrae from these recaptured animals need to periodically processed in the laboratory, and the results of statistical analysis of the data need to be periodically updated and reported. The laboratory and data analysis procedures have all been developed; periodic updates will require only a limited amount of work. These tasks now form part of the Southern Shark Monitoring Project funded by AFMA through industry levies.

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Intellectual property

No intellectual property has arisen from the research that is likely to lead to significant commercial benefits, patents, or licences. Intellectual property associated with information produced from the project will be shared equally by the Fisheries Research and Development Corporation and by the Victorian Department of Natural Resources and Environment.

Staff

Organisation, position, period on the project and percentage of time each year on the project are listed for each staff member.

Marine and Freshwater Resources Institute			
Terence I. Walker	Principal Investigator	1 Jul 97–30 Jun 00	10%
Lauren P. Brown	Marine Scientist	1 Jul 97–30 Jun 00	25%
School of Oral Medicine, The University of Melbourne			
John G. Clement	Collaborative Investigator	1 Jul 97–30 Jun 00	5%

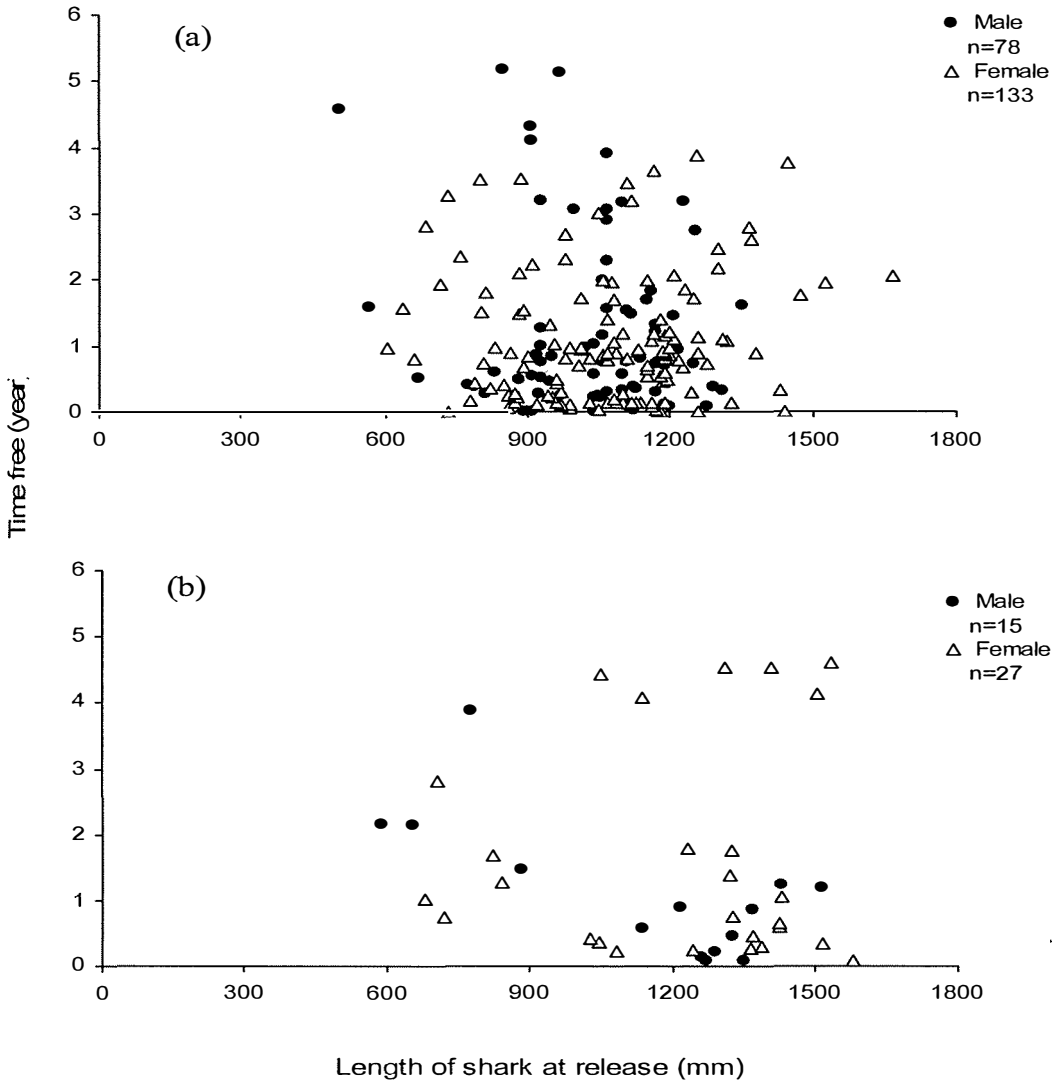
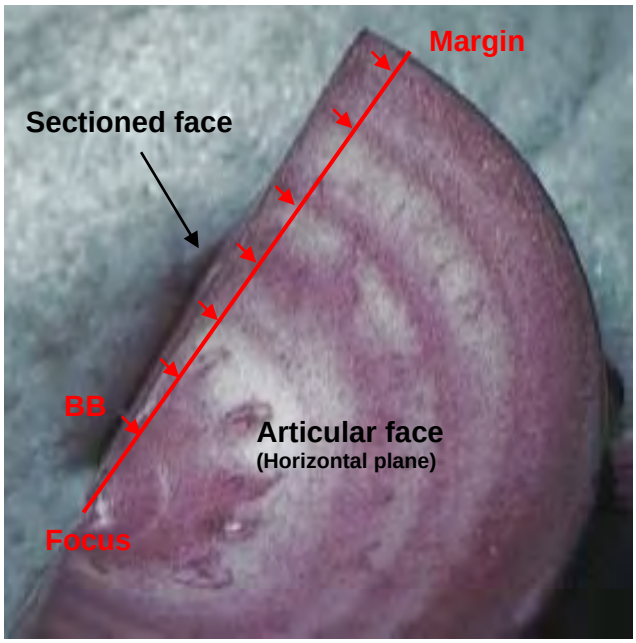
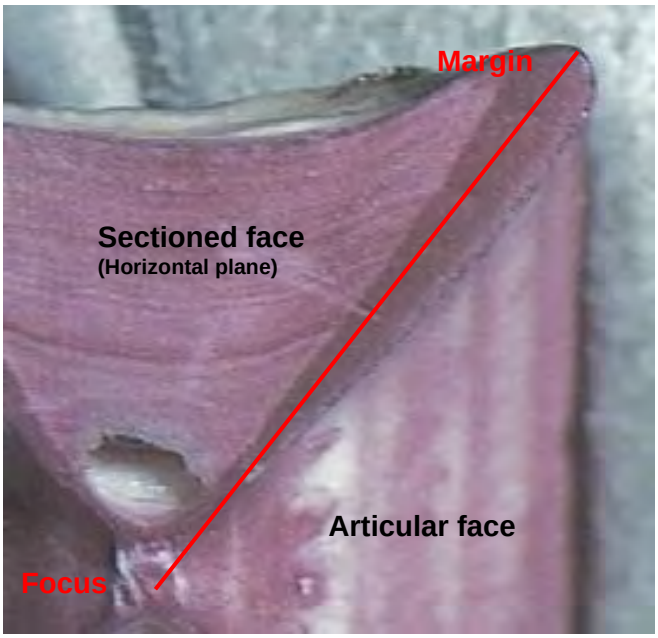


Figure 1. Time free and length of shark for recaptured oxytetracycline injected sharks

(a) 'Transect' and AS1 series of measurements

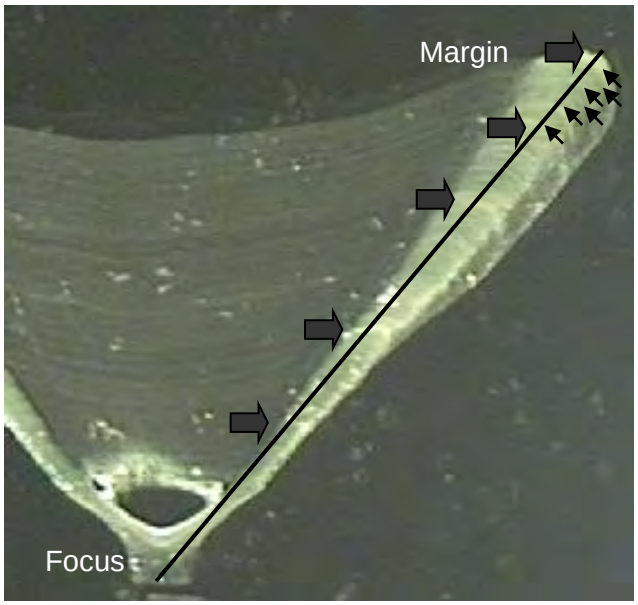
(b) 'Transect' and AS2 series of measurements

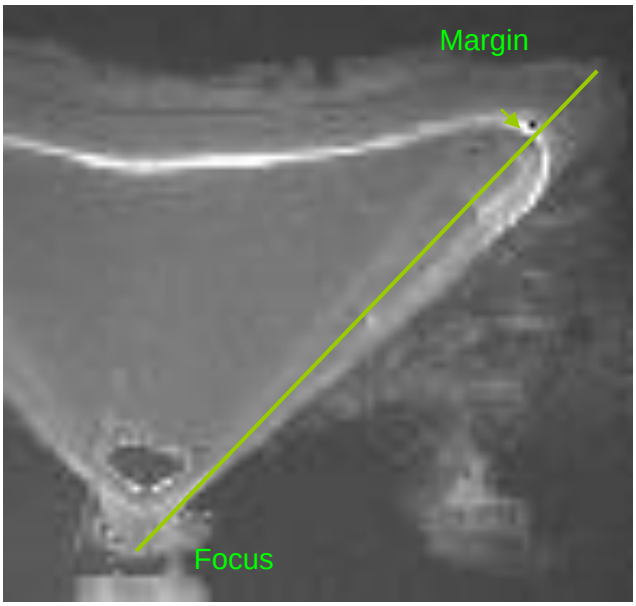




(c) 'Transect' and MR1 and MR2 series of measurements

(d) 'Transect' and OM1 series of measurements





(e) Standardized measurements AS1, MR1, MR2, and OM1 with counts and readabilities R

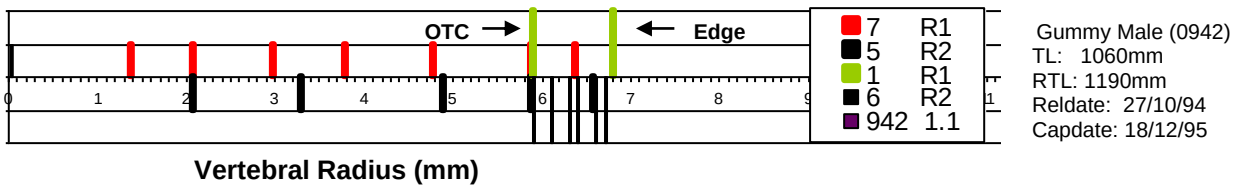


Figure 2. Diagrammatic representation of band measurements

Measurements taken from the alizarin stained vertebra-half (a and b), the microradiograph of the 100 µm thick section (c), and fluorescent image of the 100µm thick section (d) of recaptured tagged male gummy shark Number 942, and standardised vertebral radius measurements of AS1, MR1, MR2, and OM1 (e). The shark grew 130 mm from 1060 mm to 1190 mm in 1.1 years, including one winter, with one AS1 (red), one MR1 (thick black) and six MR2 (thin black) growth increment bands observed outside the oxytetracycline mark, OM1 (green).

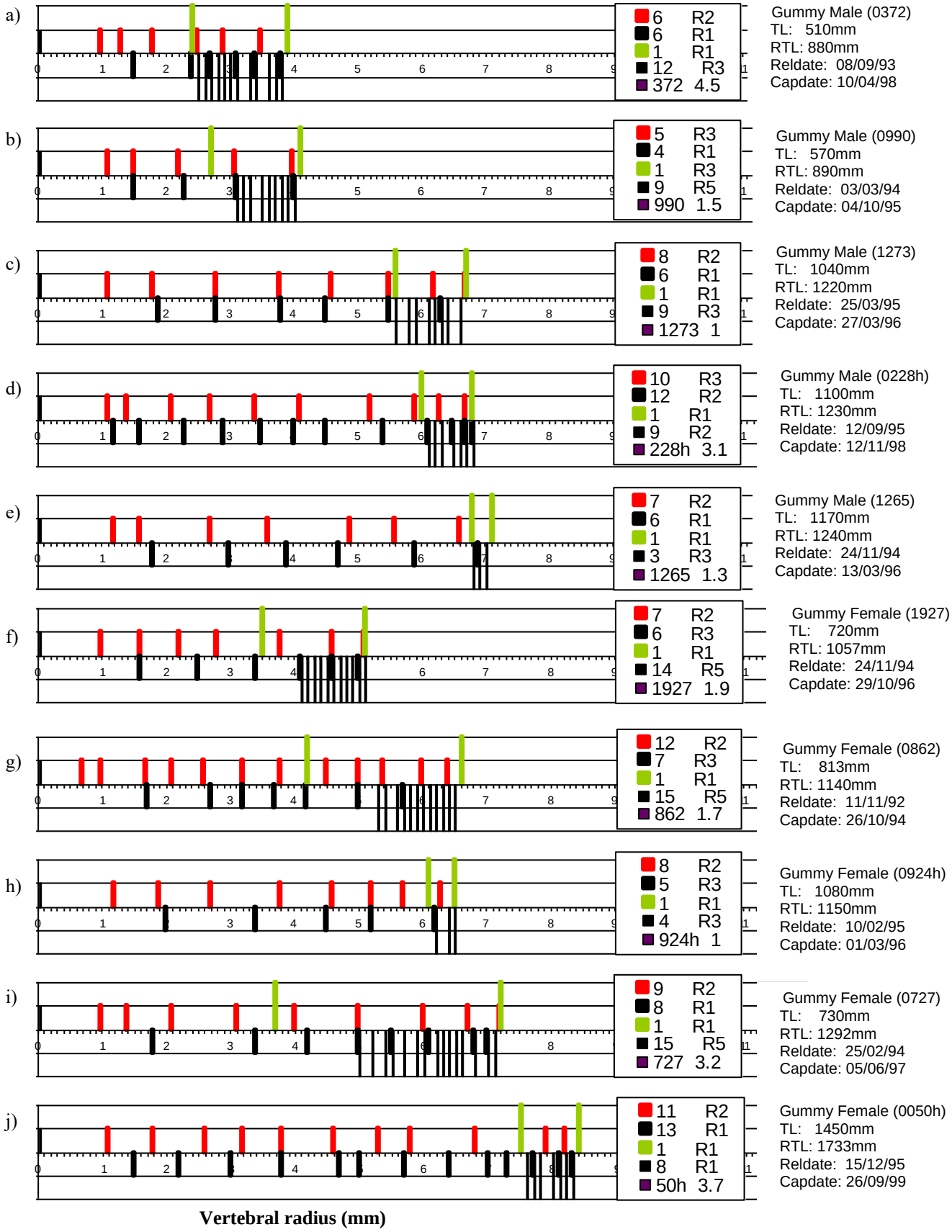


Figure 3a Standardised band measurements for selected gummy sharks

Measurements are from the Alizarin Staining Method and the Microradiograph Method relative to the oxytetracycline mark on postcranial vertebrae. AS1, red; MR1, thick black; MR2, thin black; OM1, green; R, readability score.

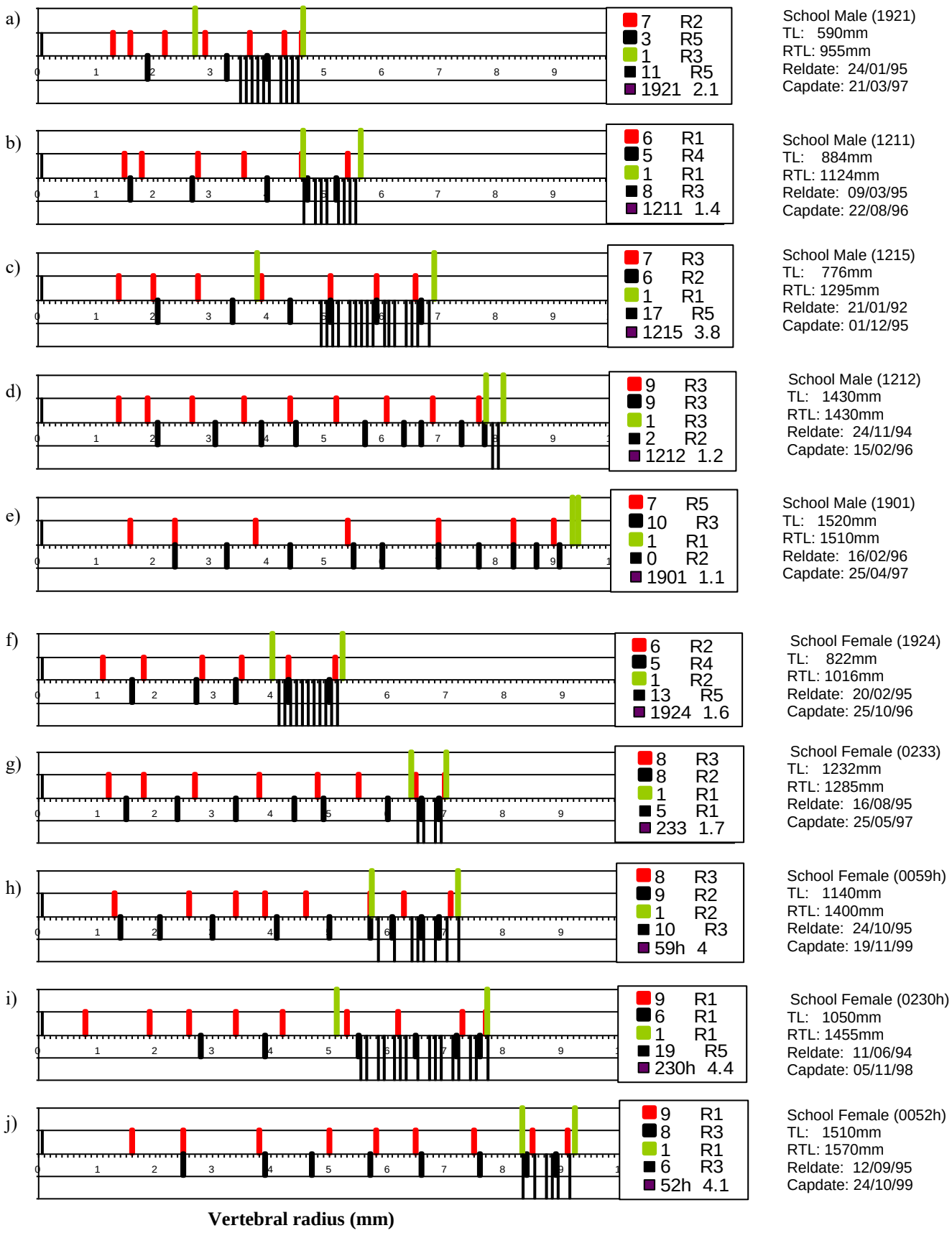


Figure 3b. Standardised band measurements for selected school sharks

Measurements are from the Alizarin Staining Method and the Microradiograph Method relative to the oxytetracycline mark on postcranial vertebrae. AS1, red; MR1, thick black; MR2, thin black; OM1, green; R, readability score.

Table 1. Band count outside the oxytetracycline mark and number of winters

Band count is the number of bands counted outside the oxytetracycline mark of a recaptured oxytetracycline injected tagged shark and the number of winters refers to the number of winters the shark was at liberty; SD, standard deviation. The length-classes 'small', 'medium', and 'large' are <1000 mm, 1000–1199 mm, and ¤ 1200 mm, respectively, for female gummy shark; <1000 mm, 1000–1099 mm, and ¤ 1100 mm, respectively, for male gummy sharks; and <1000 mm, 1000–1399 mm, and ¤ 1400 mm, respectively, for both female and male school shark.

Ageing method	Species	Sex	Length-class	Sample size	No. bands	No. winters	Mean bands per winter
					Mean (SD)	Mean (SD)	
Alizarin Staining	Gummy shark	Female	Small	14	2.50 (1.35)	2.07 (0.83)	1.21
			Medium	11	1.91 (1.14)	1.82 (1.17)	1.05
			Large	16	1.94 (1.00)	2.13 (0.96)	0.91
			Sub-total	41	2.12 (1.17)	2.02 (0.96)	1.05
		Male	Small	9	2.33 (1.23)	3.22 (1.56)	0.72
			Medium	9	1.33 (0.71)	2.44 (1.01)	0.55
			Large	9	1.11 (0.93)	1.44 (0.73)	0.77
			Sub-total	27	1.59 (1.08)	2.37 (1.33)	0.67
		Total	Small	23	2.44 (1.27)	2.52 (1.28)	0.97
			Medium	20	1.65 (0.99)	2.10 (1.12)	0.79
			Large	25	1.64 (1.04)	1.88 (0.93)	0.87
			Sub-total	68	1.91 (1.16)	2.16 (1.13)	0.88
	School shark	Female	Small	4	2.00 (0.82)	1.75 (0.96)	1.14
			Medium	6	2.00 (1.27)	3.00 (1.55)	0.67
			Large	4	1.25 (0.96)	3.25 (1.50)	0.38
			Sub-total	14	1.79 (1.05)	2.71 (1.44)	0.66
		Male	Small	3	3.00 (1.73)	2.33 (1.53)	1.29
			Medium	0	— (—)	— (—)	—
			Large	1	0.00 (—)	1.00 (—)	0.00
			Sub-total	4	2.25 (2.06)	2.00 (1.41)	1.13
		Total	Small	7	2.43 (1.27)	2.00 (1.16)	1.22
			Medium	6	2.00 (1.27)	3.00 (1.55)	0.67
			Large	5	1.00 (1.00)	2.80 (1.64)	0.36
			Sub-total	18	1.89 (1.28)	2.56 (1.42)	0.74
Microradiographic	Gummy shark	Female	Small	15	2.73 (1.49)	2.00 (0.85)	1.37
			Medium	11	2.09 (1.22)	1.81 (1.17)	1.15
			Large	15	2.00 (1.51)	2.00 (0.85)	1.00
			Sub-total	41	2.93 (1.44)	1.95 (0.92)	1.50
		Male	Small	9	2.56 (1.42)	3.22 (1.56)	0.80
			Medium	9	2.00 (1.41)	2.44 (1.01)	0.82
			Large	9	1.56 (1.51)	1.44 (0.73)	1.08
			Sub-total	27	2.04 (1.45)	2.37 (1.33)	0.86
		Total	Small	24	2.67 (1.44)	2.46 (1.29)	1.09
			Medium	20	2.05 (1.28)	2.10 (1.12)	0.98
			Large	24	1.83 (1.49)	1.79 (0.83)	1.02
			Sub-total	68	2.19 (1.44)	2.12 (1.11)	1.03
	School shark	Female	Small	3	2.00 (0.00)	1.33 (0.58)	1.50
			Medium	6	2.67 (0.82)	3.00 (1.55)	0.89
			Large	4	0.75 (0.96)	3.25 (1.50)	0.23
			Sub-total	13	1.92 (1.12)	2.69 (1.49)	0.71
		Male	Small	2	3.00 (1.41)	2.50 (2.12)	1.20
			Medium	0	— (—)	— (—)	—
			Large	2	0.00 (0.00)	1.00 (0.00)	0.00
			Sub-total	4	1.50 (1.92)	1.75 (1.50)	0.86
		Total	Small	5	2.40 (0.89)	1.80 (1.30)	1.33
			Medium	6	2.67 (0.82)	3.00 (1.55)	0.89
			Large	6	0.50 (0.84)	2.50 (1.64)	0.20
			Sub-total	17	1.82 (1.29)	2.47 (1.51)	0.74

Table 2. Difference between band count and number of winters

Band count is the number of growth-increment bands counted outside the oxytetracycline mark in the vertebra of a recaptured oxytetracycline injected tagged shark and the number of winters refers to the number of winters the shark was at liberty.

Ageing method	Species	Sex	Number of bands minus number of winters							Total
			<–2	–2	–1	0	+1	+2	>+2	
Alizarin Staining	Gummy shark	Female	0	5	5	14	15	2	0	41
		Male	2	3	11	9	2	0	0	27
		Total	2	8	16	23	17	2	0	68
	School shark	Female	1	3	5	4	1	0	0	14
		Male	0	0	1	2	0	1	0	4
		Total	1	3	6	6	1	1	0	18
Microradiographic	Gummy shark	Female	1	2	8	10	13	5	2	41
		Male	2	2	6	11	4	2	0	27
		Total	3	4	14	21	17	7	2	68
	School shark	Female	2	1	4	3	3	0	0	13
		Male	0	0	2	1	1	0	0	4
		Total	2	1	6	4	4	0	0	17

Table 3. Model selection for testing hypothesis of annual periodicity of band deposition

Df, degrees of freedom; *P<0.05; **P<0.01; ***P<0.001; ns, not significant. For all generalised linear models, an error-structure with a normal probability distribution was adopted using the SAS GENMOD procedure. All models converged.

Ageing method	Species	Model	Model factors and interaction terms	df	Scaled Pearson χ^2/df	Log likelihood
Alizarin Staining	Gummy shark	1	Sex**, Length-class^{ns}, Sex x length-class^{ns}	62	1.097	–55.039
		2	Sex**, Length-class^{ns}	64	1.063	–55.430
		3	Sex**	66	1.030	–55.867
		4	Nil	67	1.015	–60.903
	School shark	5	Length-class***	15	1.200	–5.292
		6	Nil	17	1.059	–14.286
Microradiographic	Gummy shark	1	Sex*, Length-class^{ns}, Sex x length-class^{ns}	62	1.097	–68.174
		2	Sex*, Length-class^{ns}	64	1.063	–69.461
		3	Sex*	66	1.030	–70.374
		4	Nil	67	1.015	–72.540
	School shark	5	Length-class***	14	1.214	–8.059
		6	Nil	16	1.063	–18.480

Table 4. Model prediction of number of bands deposited per winter

Values of the number of growth-increment bands predicted by generalised linear models using the SAS GENMOD procedure. SE, standard error; CI, confidence interval.

Ageing method	Species	Sex	Length-class	Sample size	Predicted number of bands deposited per winter		
					Mean	SE	95% CI
Alizarin Staining	Gummy shark	Female		41	1.17	0.17	0.82–1.51
		Male		27	0.72	0.11	0.51–0.92
		Combined		68	0.99	0.07	0.85–1.13
	School shark	Combined	Small	7	1.29	0.24	0.82–1.75
			Medium	6	0.68	0.24	0.20–1.15
			Large	5	0.25	0.15	0.00–0.53
			Sub-total	18	0.79	0.13	0.55–1.04
Microradiographic	Gummy shark	Female		41	1.27	0.21	0.85–1.69
		Male		27	0.91	0.13	0.65–1.17
		Combined		68	1.12	0.09	0.96–1.29
	School shark	Combined	Small	5	1.60	0.28	1.04–2.16
			Medium	6	1.05	0.27	0.51–1.59
			Large	6	0.13	0.16	–0.19–0.44
			Sub-total	17	0.89	0.17	0.54–1.23