Received: 12 September 2017 | Accepted: 21 December 2017

DOI: 10.1111/jai.13620

ORIGINAL ARTICLE

WILEY Journal of Applied Ichthyology DWK

Development of an alternative ageing technique for blue shark (*Prionace glauca*) using the vertebra

Y. Fujinami[1,2] | Y. Semba[1] | S. Ohshimo[3] | S. Tanaka[2]

[1]National Research Institute of Far Seas Fisheries, Japan Fisheries Research and Education Agency, Shizuoka, Japan

[2]School of Marine Science and Technology, Tokai University, Shizuoka, Japan

[3]Seikai National Fisheries Research Institute, Japan Fisheries Research and Education Agency, Nagasaki, Japan

Correspondence
Yuki Fujinami, National Research Institute of Far Seas Fisheries, Japan Fisheries Research and Education Agency, Shizuoka, Japan.
Email: y.fuji925@gmail.com

Summary

Accurate determination of age is essential for the comprehensive assessment and management of exploited shark populations. Enhancement of growth bands is necessary to accurately and efficiently determine age. However, most traditional techniques do not describe an efficiency of a series of procedures and the detailed protocol for different-sized sharks. We describe a simple and highly successful technique for ageing vertebrae of blue shark (*Prionace glauca*) that we refer to as "burn method", derived from the "break and burn method" and "shadowing method"—a simple procedure requiring an alkaline treatment to clean the vertebral centra and a burning treatment to enhance growth band visualization. We described optimal times for sharks of precaudal length of 50, 100, 150, and 200 cm as 44.9, 88.7, 134.0, 183.5 s of alkaline treatment, and 6.8, 8.9, 10.2, and 11.5 min of burning treatment; both longer and shorter exposure times resulted in higher failed sample frequencies. Using our burn method, it is possible to do ageing a sample from a wide angle, with the reader able to obtain information on growth bands from different perspectives. To critique different technique efficiencies, the index of average percent error (IAPE) and mean coefficient of variation (CV) are compared for independent readers using the burn method and other techniques (silver nitrate impregnation and the unstained shadowing methods). The precision of the burn method (IAPE, 4.1%; CV, 5.7%) was similar to that of silver nitrate impregnation (IAPE, 5.8%; CV, 8.2%) and unstained shadowing method (IAPE, 8.3%; CV, 11.8%). For younger specimens, the IAPE and CV of the burn method were lower than those of the other techniques, but, compared with other ageing methods, precision decreased for older sharks. We demonstrate that the burn method to be accurate for age determination of blue sharks, especially for specimens with less than 10 bands, but recommend simultaneous use of other methods, such as thin sectioning (the most common for structure-based ageing) and bomb carbon dating, to accurately determine the age of older individuals.

1 | INTRODUCTION

Accurate age determination is essential for the comprehensive assessment and management of exploited shark populations (Ricker, 1975), as information about age and growth is required to estimate key biological parameters, such as growth rate, mortality rate, and productivity (Campana, 2001). Elasmobranchs lack the hard elements, such as otoliths and scales, commonly used to determine age of teleost fish (Cailliet, Martin, Kusher, Wolf, & Welden, 1983). Therefore, the age of elasmobranchs is typically estimated by counting growth bands that form on hard structures, such as vertebrae and dorsal fin spines (Goldman, Cailliet, Andrews, & Natanson, 2012; Matta, Tribuzio, Ebert,

Goldman, & Gburski, 2017). Several techniques that enhance the visibility of growth bands on the vertebral centra to improve the accuracy of age determination have been used in elasmobranchs; these include staining of whole- or thin-sectioned centra (e.g., silver nitrate [Nakano, 1994; Stevens, 1975], hematoxylin [Tanaka, Cailliet, & Yudin, 1990]), histology (Casey, Pratt, & Stillwell, 1985), shadowing (Francis & Maolagáin, 2000; Oshitani, Nakano, & Tanaka, 2003; Semba, Nakano, & Aoki, 2009), and radiography (Cailliet et al., 1983; Wells, Spear, & Kohin, 2017). These traditional techniques provide clear images, but most of them typically require time-consuming sample preparation and substantial experimental practice and experience (e.g., Cailliet et al., 1983; Campana, 2001). Of these methods, thin sectioning is the most common in studies of elasmobranch age and growth (Goldman et al., 2012; Matta et al., 2017; though this method can lead to fuzziness in growth bands in cross-sections of the *corpus calcareum* of younger specimens (Tanaka, Kitamura, Mochizuki, & Kofuji, 2011).

The blue shark (*Prionace glauca*) is a large pelagic species that is widely distributed in tropical, subtropical, and temperate waters, and is a valuable fisheries resource caught by pelagic longline fisheries as both target catch and bycatch (Nakano & Stevens, 2008). The second most recent stock assessment of blue sharks in the North Pacific was conducted by the International Scientific Committee for Tuna and Tuna-like Species in the North Pacific Ocean (ISC, 2014), with the growth parameters for assessment based on estimates from specimens collected from 1982 to 1983. As the growth coefficient of some sharks varies depending on density (e.g., Carlson & Baremore, 2003; Sminkey & Musick, 1995), a decline in populations could lead to faster growth and earlier maturity than conditions of a larger population size (Carlson & Baremore, 2003; Sminkey & Musick, 1995). North Pacific blue shark stocks have been increased since the 1980s (ISC, 2014), indicating growth parameters might have changed from those used for the last stock assessment. Therefore, re-estimation of the growth parameters for blue sharks in the North Pacific was considered priority research for improvement of stock assessments (ISC, 2014).

Estimates of growth parameters are influenced by ageing technique, sample size, and sample bias (Tanaka et al., 1990). To reduce these effects, adequate samples encompassing the shark's habits, size, and sexes are needed. As blue sharks are widely distributed throughout the North Pacific, and their movement pattern changes with sex (Nakano, 1994), a large number of samples require analysis. Enhancing the efficiency of ageing techniques therefore would increase the number of samples that could be processed, thereby improving the accuracy of age and growth information for reliable stock assessment and management. Accordingly, we developed an alternative ageing technique to improve the efficiency of sample processing, enabling a large number of samples to be processed quickly.

The break and burn method is commonly used to enhance growth bands on teleost fish otoliths (e.g., Christensen, 1964; Ohshimo et al., 2014). This method is simple and cost-effective, as the otoliths are removed and burned using an alcohol burner or a drying oven. For elasmobranch vertebrae, the shadowing method (Francis & Maolagáin, 2000) is simple, effective, relative fast, and can provide readers with information on the growth bands from wide angles and perspectives. However, blue shark vertebrae are difficult to read because the poor

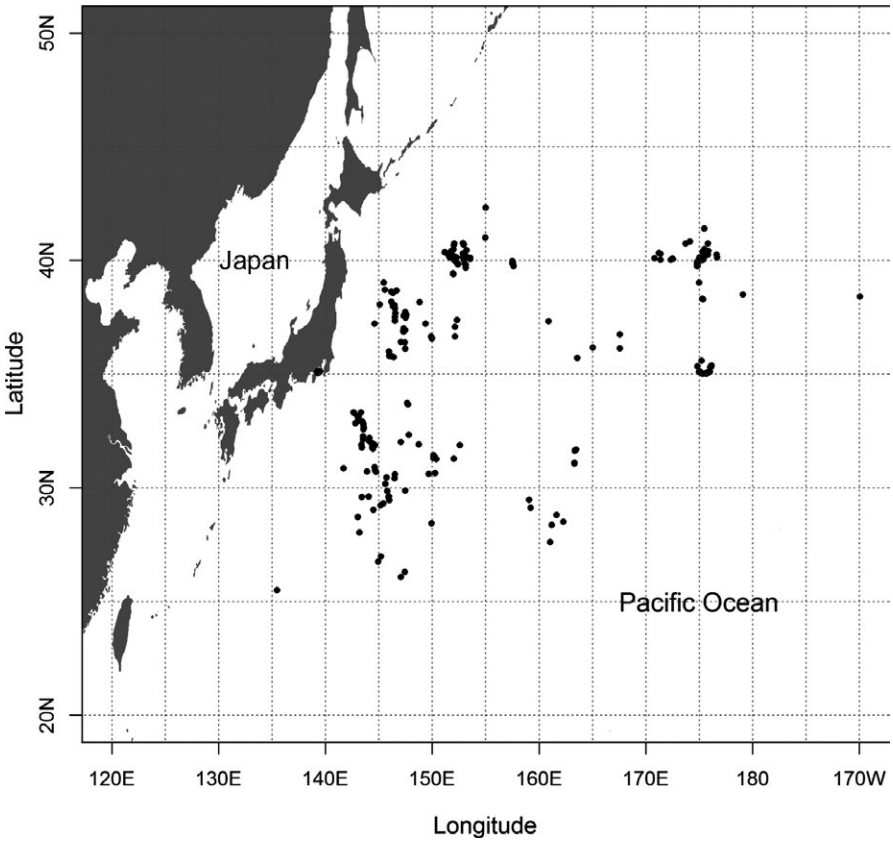

FIGURE 1 Sampling locations for *Prionace glauca* in the western North Pacific Ocean

contrast between opaque and translucent growth bands (e.g., Jolly, da Silva, & Attwood, 2013; Skomal & Natanson, 2003). Hence, we modified the break and burn method and shadowing method, referring to the revised method as the "burn method" to blue shark vertebrae. This technique requires an initial alkaline treatment of the vertebra to remove connective tissue from the vertebral centra, and a subsequent burning to enhance the growth band pair.

The purpose of the present study was to develop a new band enhancement technique, particularly to: (i) provide the optimal exposure time for the alkaline and burning treatment, and (ii) establish the efficacy of the burn method for age determination using the vertebral centra of blue sharks from the western North Pacific Ocean.

2 | MATERIALS AND METHODS

2.1 | Sample collection and burn method procedures

Blue sharks were captured from 2010 to 2015 by Japanese research vessels and commercial long-liners operating in the western North Pacific Ocean (Figure 1). Precaudal length (PCL) and dorsal length (DL: length from the origin of the first dorsal fin to the origin of the second dorsal fin) in a natural position were measured to the nearest cm for samples collected by the research vessels. Only DL was measured for sharks caught by the commercial vessels, as the head and viscera had been removed before samples were landed at fish markets. Thus, DL was converted to PCL using the conversion formula detailed in Fujinami, Semba, Okamoto, Ohshimo, and Tanaka (2017).

Vertebral samples (n = 400; 33.4–258.3 cm PCL) were excised from the region above the branchial chamber and stored frozen prior to processing. In the laboratory, the vertebral samples were boiled for approximately 20 min to remove most connective tissue, and the vertebral centra were stored in 70% ethanol. The burn method used in the present study employed two steps as follows:

2.1.1 | Step 1: alkaline treatment

Alkaline treatments were conducted according to Semba et al. (2009). The vertebral centra were washed in running water and soaked in sodium hydroxide solution (5 N NaOH) at 50°C. A polishing buff (8-inch buff micro-cloth; Sankei Co, Tokyo, Japan) was used to scrub connective tissue from the vertebral centrum surface. In general, longer soaking durations in the alkaline solutions (e.g., sodium hypochlorite solution) were necessary for larger centra (Goldman et al., 2012), given the amount of connective tissue on the centrum surface increased with centra size, we exposed tissues to NaOH for each 10-cm size classes for 30–240 s durations, then evaluated their condition as a success or failure. The treatment was regarded as successful if all the following criteria were satisfied:

1. Connective tissue was completely removed from the centrum surface (Figure 2a,b).
2. Convex and concave structures could be observed clearly (Figure 2a,c).

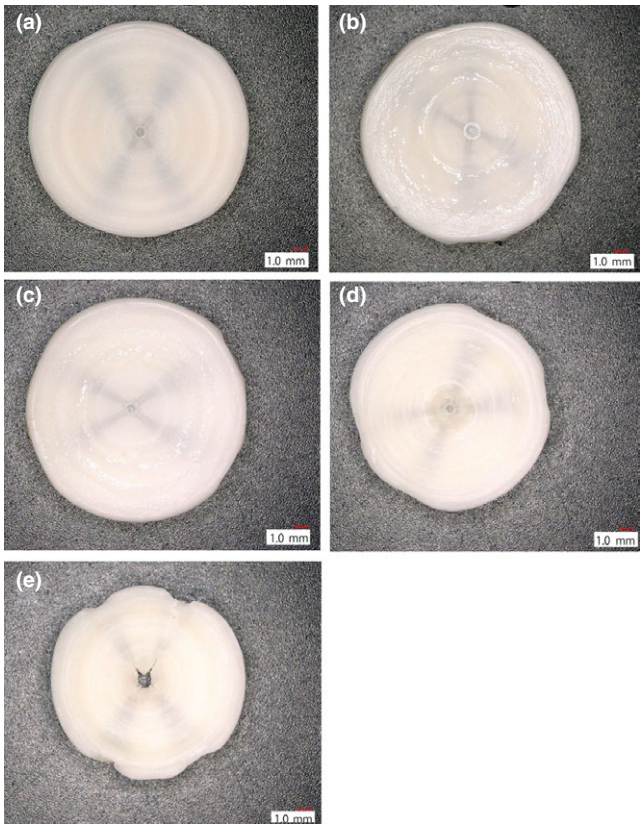

FIGURE 2 Images of vertebral centra during each step of alkaline treatment: (a) successful sample; (b) centra before exposure; (c) failed sample with connective tissue still attached after a short exposure time; (d) failed sample with the centrum surface and edge dissolved after a long exposure time; and (e) failed sample with the centra deformed after a much longer exposure time

3. The edge structures of the vertebral centrum were not dissolved (Figure 2a,d,e).

Following the alkaline treatment, the samples were washed in running water, cut into two longitudinal sections using a diamond saw (Minilab-cutter, MC110; Maruto Instrument Co, Tokyo, Japan) with the focus slightly to one side, and the half-cut centrum with the focus air-dried for 1 day.

2.1.2 | Step 2: burning treatment

Following Step 1, half-cut centra were heated at 250°C in a drying oven (DO-300A; AS-ONE, Osaka, Japan), for 2–30 min. Success was achieved if the following two criteria were met:

1. The centrum surface and edge were not charred (Figure 3a,b,d).
2. The centrum surface and edge were browned (Figure 3a,c,d).

The burned half-cut centra were observed with reflected light from both sample sides using a digital microscope and fiber-optic light (VH-8000; Keyence Co, Osaka, Japan), following Francis and Maolagáin (2000) and Semba et al. (2009).

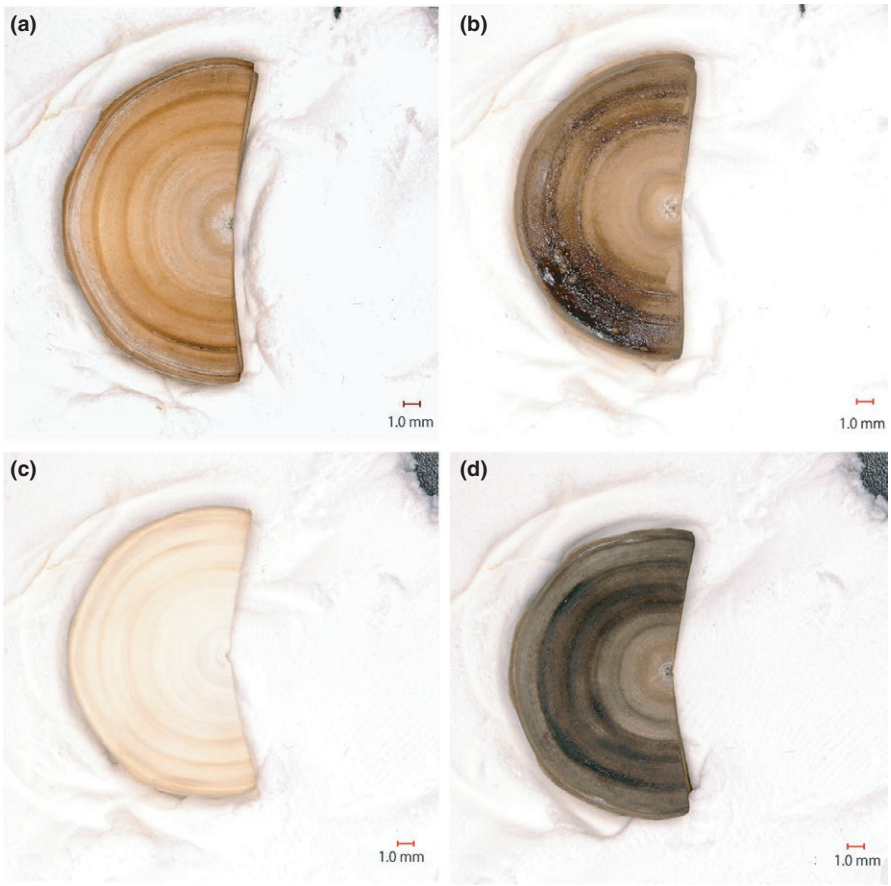

FIGURE 3 Images of vertebral centra during burning treatment: (a) successful sample; (b) failed sample with shorter alkaline exposure time; centrum surface with remaining connective tissue charred; (c) failed sample with centrum surface and edge uncolored, with less contrast due to a short burning treatment; (d) failed sample with charred centra after a longer exposure time

2.2 | Estimate of optimal exposure times

Generalized linear models (GLM) were used to ascertain a series of optimal alkaline and burning exposure times for various blue shark body sizes. This model approach can be used to predict suitable exposure times for untested conditions. The probability of success was defined as the response variable assuming a binomial error distribution:

$$Y_i \cong \mathrm{Bin}\left(1, \pi_i\right),$$

$$\mathrm{var}\left(Y_i\right) = \pi_i \times \left(1 - \pi_i\right),$$

$$\mathrm{Logit}\left(\pi_i\right) \cong \alpha + \mathrm{PCL} + \mathrm{Time} + \mathrm{PCL} \times \mathrm{Time},$$

where Y_i is the response variable for the success or failure of a specimen i; Bin,π_i, and α represent binomial error, the probability of success, and a constant, respectively. PCL, Time, and PCL × Time represent the PCL of specimen i, exposure time, and interaction between PCL and time; these are continuous variables. In addition, PCL and Time were assumed to be quadratic polynomials because they have a single peak. After the GLMs were established, we predicted the probability of success with a matrix of fine scale time and PCL combinations using the coefficients for each covariate to indicate the optimal exposure time according to PCL. For model validation, we applied randomized quantile residuals using the STATMOD package in R software to verify normality. All analyses were conducted using R software (version 3.3.0; R Development Core Team, 2016).

2.3 | Comparative methods

To evaluate the accuracy of the age estimate determined by the burn method, 119 centra from the same specimens were analyzed using three techniques: (i) the burn method, (ii) silver nitrate impregnation (whole centra), an alternative and efficient staining technique (Başusta, Demirhan, Çiçek, & Başusta, 2017) often used for age determination of blue sharks (e.g., Nakano, 1994; Stevens, 1975), and (iii), the unstained shadowing method. The procedure using silver nitrate impregnation followed Stevens (1975), with the total exposure time for silver nitrate impregnation being 30–40 min per sample, excluding drying time. The procedure using the unstained shadowing method (half-cut centra) was conducted according to Francis and Maolagáin (2000), Oshitani et al. (2003) and Semba et al. (2009), with the total exposure time approximately 10 min per sample, excluding drying time.

2.4 | Evaluation of precision

Clay was positioned between the centra and the microscope stage to adjust the observation angle. One pair of convex and concave structures was defined as a growth band pair, and the number of convex structures was counted for both the burn and unstained shadowing methods. Whole centra that were silver nitrate impregnated were observed under a digital microscope. Using this method, we defined one pair of opaque and translucent bands as a growth

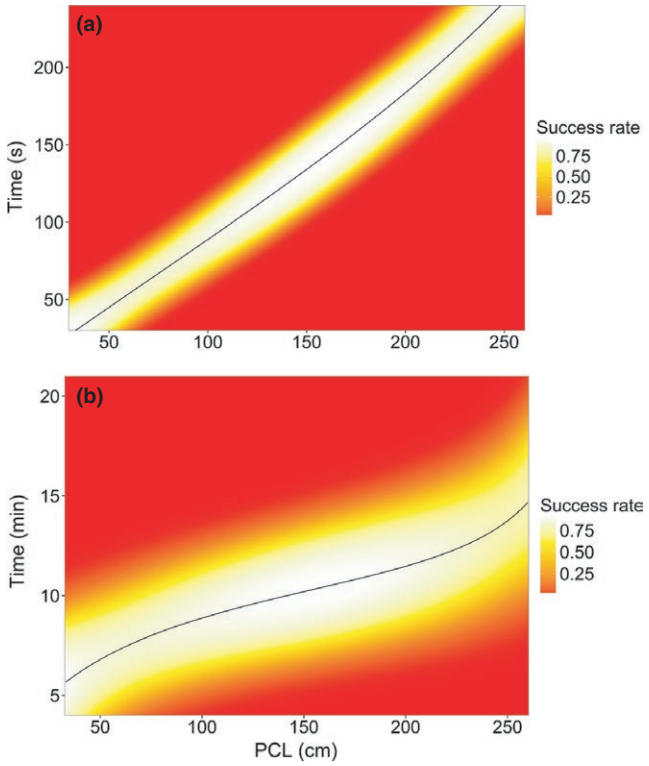

FIGURE 4 Predicted possibility of success according to precaudal length (PCL, cm) and exposure time for (a) alkaline treatments (s), and (b) burning treatments (min), based on the generalized linear model (GLM). Solid lines show the optimal exposure time with the highest probability of success estimated by GLM

band pair, and counted the number of translucent bands. For each method, the number of growth bands was counted by two separate readers, without prior knowledge of a specimen's length or the band counts of the second reader. The index of average percent error (IAPE) (Beamish & Fournier, 1981) and mean coefficients of variation (CV) (Chang, 1982) were calculated, and age-bias plots (Campana, Annand, & McMillan, 1995) were constructed to assess the bias between reader counts for each technique. Differences in growth band counts between readers were tested using the Wilcoxon signed-ranks test.

3 | RESULTS

3.1 | Optimal exposure time of the burn method

A total of 400 individuals of PCL 33.4–258.3 cm were examined 642 alkaline treatment trials and 753 burning treatment trials were used for GLM analysis (Tables S1 and S2). Bands were enhanced successfully in all individuals (success of both the alkaline and burning treatments) under the particular exposure times. Model convergence was achieved, and all explanatory variables were statistically significant for both the alkaline and burning treatment models (Tables S3 and S4). The histogram of randomized quantile residuals for the alkaline and burning treatments was close to being normally distributed (Figure S1). The optimal exposure time, defined as the time resulting in the highest probability of success within the same body size, showed an increasing trend with increasing body size (Figure 4; solid lines). The optimal exposure times and acceptable range for the probability of a 75% success rate for sharks with PCLs of 50, 100, 150, and 200 cm were estimated to be 44.9 ± 26.0, 88.7 ± 28.5, 134.0 ± 38.0, and 183.5 ± 36.4 s for the alkaline treatment (case of 5 N NaOH), and 6.8 ± 3.2, 8.9 ± 3.4, 10.2 ± 4.2, and 11.5 ± 4.0 min for the burning treatment, respectively. Although the optimal exposure time increased as shark size increased, the relationship between body size and optimal exposure time in the burning treatment was weaker than that it was for the alkaline treatment. The shorter for alkaline exposure times resulted in connective tissue remaining on the centrum surface (Figure 2c), and the longer exposure time lead to dissolution of the centrum margin (Figure 2d,e). Similarly, the shorter exposure time for the burning treatment resulted in uncolored centra (Figure 3c) and the longer exposure time charred the centra (Figure 3d). Exposure times for the burning treatment exceeding 20 min were unsuccessful. The burn method enabled successful processing of one specimen within 15–20 min (excluding drying time), and batch processing of 20–30 samples at once, the number limited largely by drying oven size.

3.2 | Evaluation of precision

The burn method produced clear contrasts between convex and concave structures (dark and light bands) on the centrum surface (Figure 3a), with growth bands observed both on the centrum surface and the *corpus calcareum* and intermedialia of cut sections (Figure 5). Similarly, the unstained shadowing method enhanced convex and concave structures on both the centrum surface and longitudinal sections, though the contrast was less than for the burn method (Figure 6a). Growth bands on the surface of centra subjected to silver nitrate impregnation were clearly distinguishable, whereas those on the centrum margin were difficult to interpret (Figure 6b), in addition, the centra exhibited excess silver salt deposits, hampering interpretation of growth bands on the centrum surface.

The number of growth bands counted using the burn method ranged from 1 to 17 (reader 1) and 1 to 15 (reader 2); from 1 to 15 (reader 1) and 1 to 12 (reader 2) for silver nitrate impregnation; and from 1 to 17 (reader 1) and 1 to 12 (reader 2) for the unstained shadowing method (Figure 7). The number of growth bands for the unstained shadowing method also varied widely compared with the other techniques, particularly for reader 1. The bias plots prepared to compare counts between readers using samples treated with the burn method revealed minimal variation around the one-to-one plot until 10 bands (<200 cm PCL), but deviated thereafter (Figure 8). Results for the silver nitrate impregnation and unstained shadowing methods deviated from the one-to-one line over five and seven band counts, however, the unstained shadowing method revealed minimal variation for individuals with more than 10 bands. The IAPE and CV values were 4.1% and 5.7% for the burn method, 5.8% and 8.2% for silver nitrate impregnation, and 8.3% and 11.8% for the unstained shadowing method, respectively. Also, the IAPE and CV values for shark smaller than 200 cm PCL were lowest in the burn method, while those for

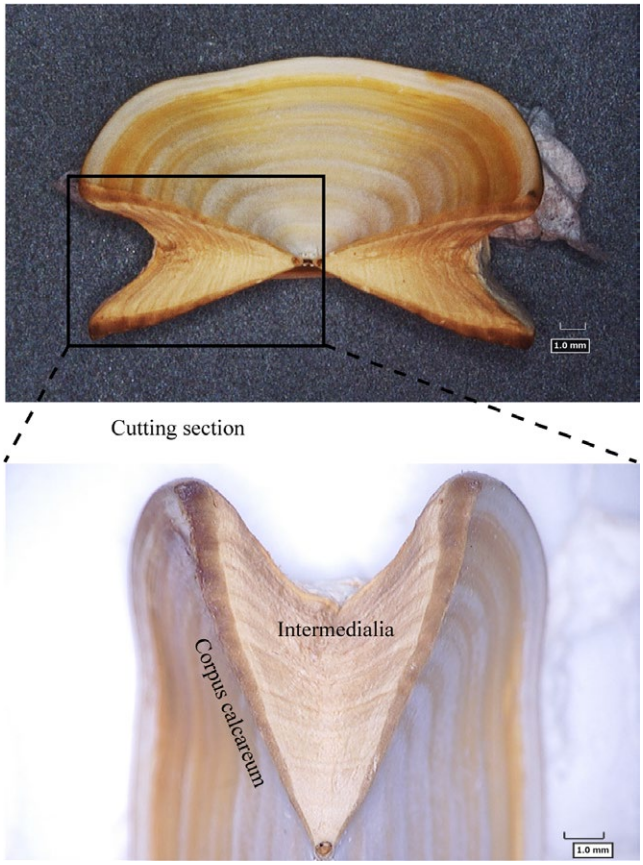

FIGURE 5 Images of centrum surface and both *corpus calcareum* and intermedialia on the cutting plane of vertebral centra treated using the burn method

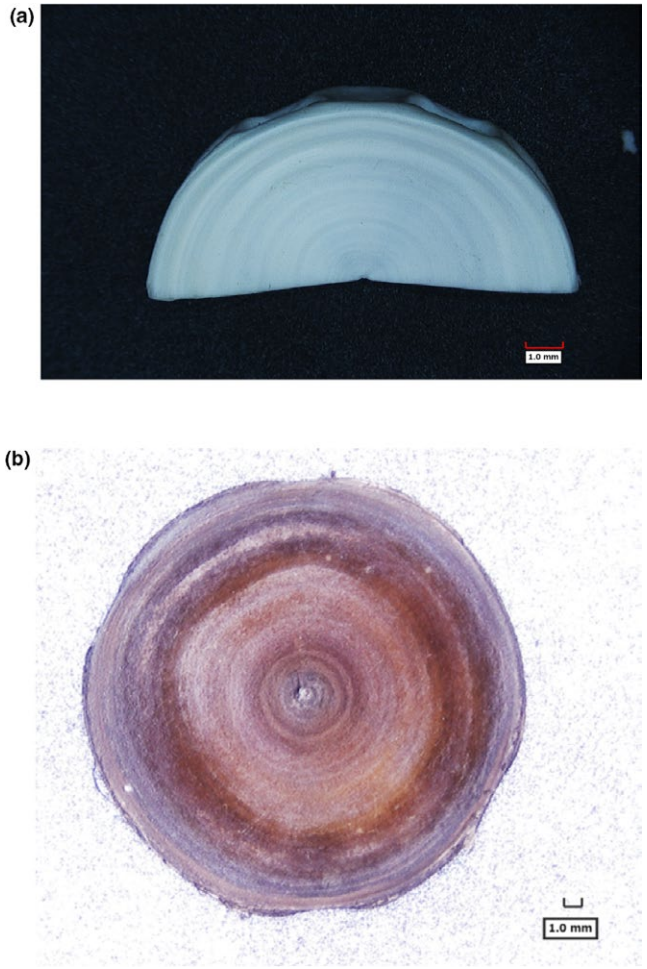

FIGURE 6 Images of vertebral centra treated by: (a) unstained shadowing method; and (b) silver nitrate impregnation

shark larger than 200 cm PCL were lowest in the unstained shadowing method (Table S5). Although there were significant differences in band counts between readers for all procedures (Wilcoxon signed-rank test, burn method; $p = .03$, silver nitrate impregnation and the unstained shadowing method; $p < .01$), no significant difference was found for only the burn method if samples were limited to fewer than 10 bands (200 cm PCL) (burn method: $p = .06$, silver nitrate and unstained shadowing method; $p < .01$).

4 | DISCUSSION

Our results indicate that the burn method can be used to accurately determine blue shark age in specimens with less than 10 bands. We found the appropriate time for each exposure treatment (alkaline and burning treatments) varied according to body size, with much longer or shorter exposure times from the optimum resulting in failed results. In addition, our results demonstrate the precision of the burn method for specimens with less than 10 bands to be relatively high compared with those analyzed using silver nitrate impregnation and unstained shadowing methods. The IAPE and CV values of the burn method (4.1% and 5.7%) were similar to those for the silver nitrate impregnation (5.8% and 8.2%) and unstained shadowing methods (8.3% and 11.8%). Also, those values for younger and smaller specimens (<200 cm PCL) were lowest in the burn method (3.0% and 4.2%), whereas those for older specimens were lowest in the unstained shadowing method (7.6% and 10.7%). The IAPE values for blue shark in other studies were 3.0% (silver nitrate [whole centra]: Blanco-Parra, Galvan-Magnna, & Marquez-Farias, 2008), 7.9% (unstained whole centra: Jolly et al., 2013), and 3.8% and 9.0% (X-ray [whole centra]: Joung, Lyu, Su, Hsu, & Liu, 2017; X-ray [thin section]: Manning & Francis, 2005). Similarly, the CVs for blue shark in other studies were 5.0% and 12.8% (X-ray [whole centra]: Joung et al., 2017; X-ray [thin section]: Manning & Francis, 2005) and 15.0% (unstained thin section: Skomal & Natanson, 2003). The IAPE and CV reference values have been reported to be 5.5% and 7.6%, respectively (Campana, 2001). The burn method had values lower than these reference levels suggests this method's accuracy is similar to other traditional methods for ageing younger specimens.

As the reader is able to examine growth bands from the centrum surface, *corpus calcareum* and intermedialia of the cut section, the burn method provides to the additional information provided to interpret the growth bands than planar sectioning method (Matta et al.,

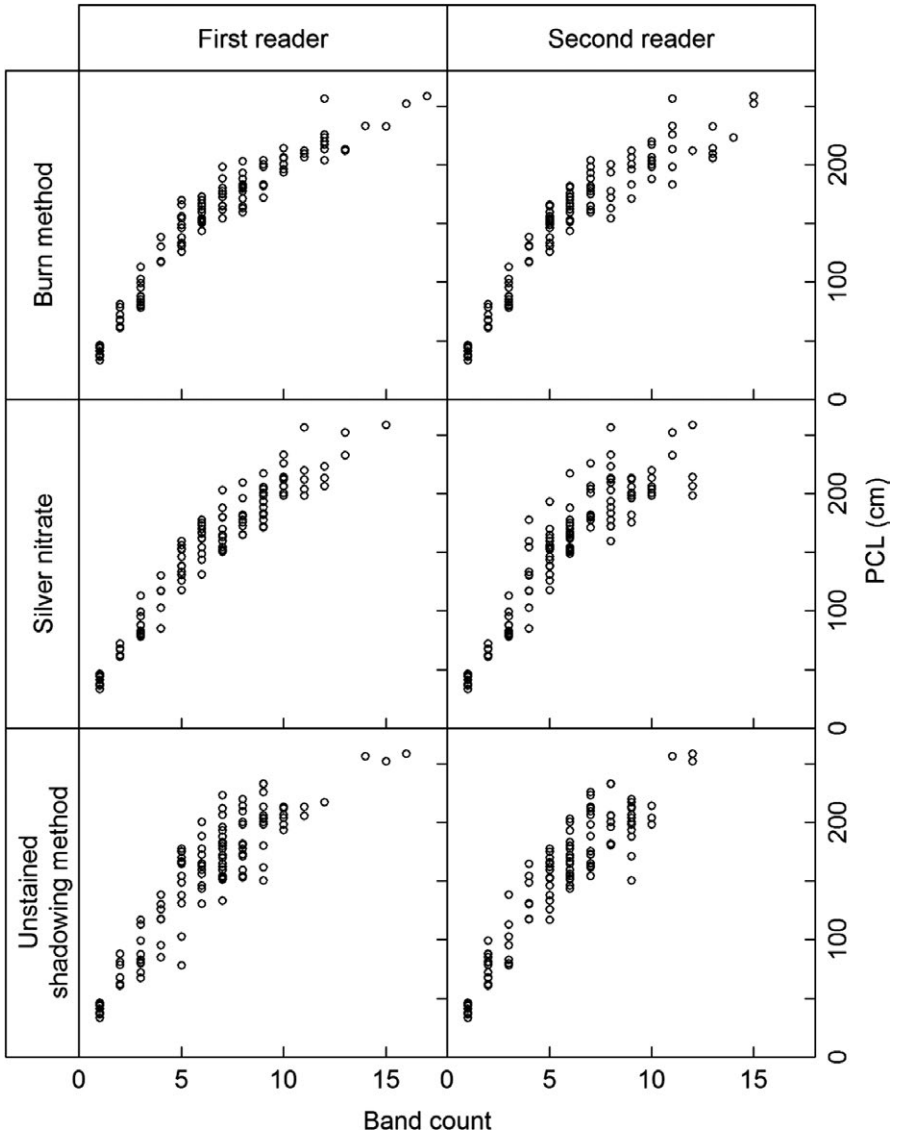

FIGURE 7 Relationship between growth band counts and precaudal length (PCL, cm) for the burn, silver nitrate impregnation, and unstained shadowing methods. First and second reader counts are shown separately for each technique

2017). Although it was also possible to age from a wide angle using the unstained shadowing method (as opposed to other techniques like thin sectioning and the silver nitrate staining), the reduced contrast between convex and concave structures (compared with those of the burn method) resulted in uncertainty of growth band interpretation, especially for younger specimens. An ageing technique using micro-computed tomography (micro-CT) has been introduced for the spinner shark *Carcharhinus brevipinna* (Geraghty, Jones, Stewart, & Macveth, 2012), such scanning produce virtual high resolution three-dimensional images of whole and half-cut centra and of sagittal sections, providing readily visible growth bands. This approach is, however, more expensive and time consuming than more-traditional methods.

In addition to enhanced accuracy, the simplicity of the burn method procedure renders the process both cost and time effective. The technique does not require expensive equipment or chemicals; only an alkaline solution (caustic soda), a drying oven, and a light equipped microscope. The only experimental procedures involved are those associated with the alkaline and burning treatments, for which we have determined appropriate exposure times to ensure high treatment success rates for different body sizes. Moreover, the burn method is particularly useful when processing large numbers of samples, given 20–30 samples can be batch processed. Thus, for the most abundant size classes of blue shark in the North Pacific, such as small and medium sharks (Nakano, 1994), this method proves efficient for specimens with less than 10 bands.

The burn method has disadvantages, such as difficulties encountered counting the growth bands of older specimens, though similar concerns have also been reported for silver nitrate impregnation (Stevens, 1975) and unstained shadowing methods (Semba et al., 2009). One reason might be the smaller sample size than that for younger individuals, but primarily because the growth zones becoming vanishingly small and unresolvable on the centrum edge of vertebrae in larger and older individuals (e.g., Campana, 2014; Harry, 2018; Natanson & Campana, 2002). The thin sectioning method is useful for detecting growth bands near the vertebral edge in older individuals for structure-based age determination methods (Campana, 2014), though current methods and structures used for ageing lead to age underestimation, especially larger and older individuals (Harry, 2018).

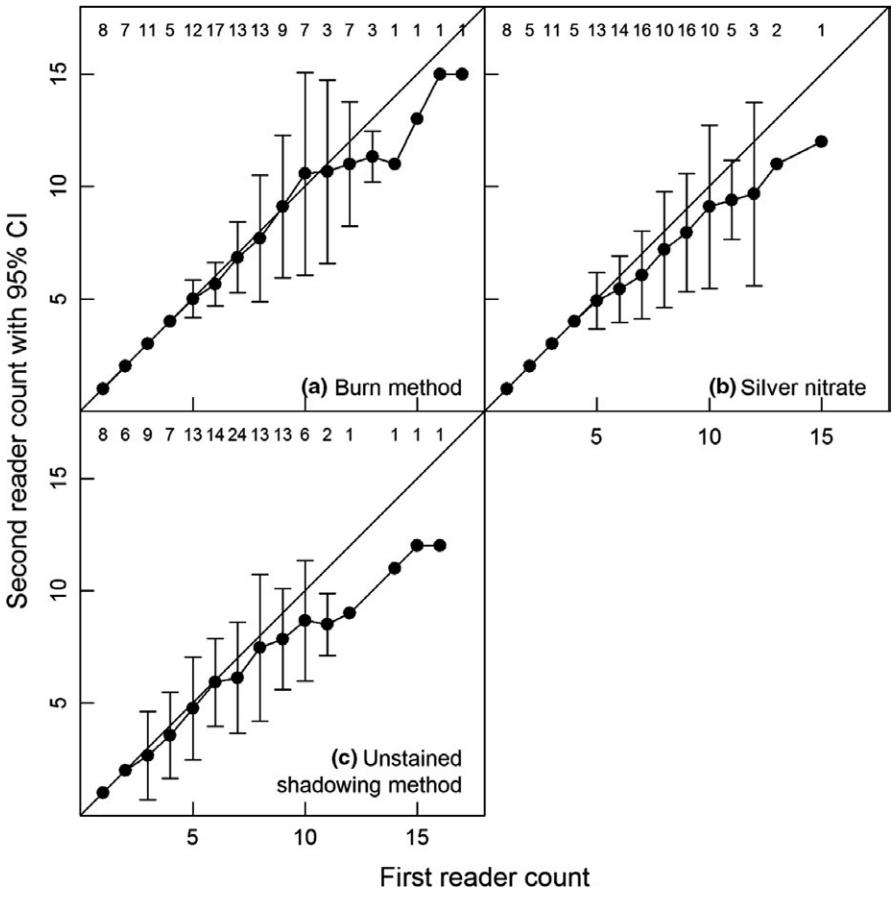

FIGURE 8 Bias-plots for: (a) burn method, (b) silver nitrate impregnation, and (c) unstained shadowing method between two readers. Each error bar represents the 95% confidence interval for the mean band count assigned by the second reader to all samples assigned a given band count by the first reader. The 1:1 equivalence line is also shown. Numbers represent sample sizes of shark

Bomb carbon dating has been recently proposed to be an effective approach for validation of elasmobranch age, especially in older individuals; of all techniques it is the most likely to give a true indication of longevity (e.g., Andrews, Natanson, Kerr, Burgess, & Cailliet, 2011; Harry, 2018; Matta et al., 2017). In conclusion, we recommend simultaneous use of the burn method (for younger individuals) combined with other methods, such as thin sectioning for structure-based age determination, and bomb carbon dating or tag-recapture dating in older individuals, to estimate growth parameters of this population.

The requirements, advantages, and disadvantages of the burn method and other traditional enhancement techniques for estimating shark age are summarized in Table 1. The burn method is particularly useful when ageing younger blue shark, in that samples are clear and can be read from wide angle, and the process is simple, cost effective, and relative quick. Some other techniques can be more complex, expensive, technologically dependent, or inaccurate (Goldman et al., 2012) although not applicable to all traditional techniques. The shadowing method is the simplest, and less time-consuming (e.g., Francis & Maolagáin, 2000). The thin-sectioning method is low cost, but the images produced are less visible without digital image enhancement (Campana, 2014) or staining. Section staining provides clear images compared with those of the thin sectioning method, but the process is relatively time consuming (Matta et al., 2017). The histological method has proved useful, but required specialized equipment and complicated processes (e.g., chemical treatments, embedding, and staining) (Goldman et al., 2012). Radiography (thin section or whole centra) has also been used in many studies (e.g., Cailliet et al., 1983; Wells et al., 2017), but an expensive X-ray machine and film-processing capabilities are required (Goldman et al., 2012). Silver nitrate impregnation (whole centra) is a low-cost method that enhances the growth bands (Stevens, 1975), but it is time consuming, additionally, if inappropriately stored, samples processed with silver nitrate do not retain clear images because of deposition of excess silver salts (Hoening & Brown, 1988), particularly for growth bands on the centrum margin should overstaining occur (Stevens, 1975). Recently, trace elemental analyses (e.g., calcium, strontium, and phosphate), such as laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), and Scanning X-ray Fluorescence Microscopy (SXFM) of vertebrae, have been applied to elasmobranchs for ageing (Hale, Dudgeon, Mason, & Lowe, 2006; Raoult et al., 2016; Scharer, Patterson, Carlson, & Poulakis, 2012); these techniques potentially allow age validation and more successful age estimation where few or no growth bands are visible.

Our study demonstrated the usefulness of the burn method for blue sharks. This method allows rapid processing in batches at relatively low cost, while maintaining accuracy levels comparable to other conventional ageing techniques. If limited to small- and medium-sized blue shark (<200 cm PCL), we consider the burn method to be an efficient vertebral ageing technique for this species and potentially other species also. Further research that compares and contrasts the

TABLE 1 Summary of band enhancement techniques for shark vertebrae

Band enhancement technique	Equipment needed	Process	Cost	Advantage	Disadvantage	Reference
Thin sectioning	Microscope, Microtome	Easy	Low	Less time consuming	Less visible without digital image enhancement or staining	Goldman et al. (2012), ISC (2012), Campana (2014), Matta et al. (2017)
Staining method (thin sectioning)	Microscope, Microtome	Relatively easy	Low	Clear images	Time consuming, difficulty counting growth bands from younger sharks	Tanaka et al. (2011), Goldman et al. (2012), ISC (2012), Matta et al. (2017)
Histology	Autotechnicon, Microtome, Microscope	Requires complicated chemical processing	High	High quality images	Time consuming, overestimates counts	Casey et al. (1985), Natanson and Cailliet (1990), Goldman et al. (2012), ISC (2012), Matta et al. (2017)
Radiography (sectioning or whole centra)	Microtome X-ray and processor	Requires film-processing capability	High	Clear images	Chemical disposal, time consuming	Cailliet et al. (1983), Goldman et al. (2012), ISC (2012)
Silver nitrate (whole centra)	Microscope	Relative easy	Low	Clear images	Chemical disposal, preservation, difficulty counting growth bands from older sharks	Stevens (1975), Hoening and Brown (1988), Goldman et al. (2012), ISC (2012)
Unstained shadowing method	Light, Microscope	Easy	Low	Less time consuming, high productivity, wide angle ageing	Difficulty counting growth bands from older sharks	Francis and Maolagáin (2000), Oshitani et al. (2003), Semba et al. (2009), ISC (2012)
Burn method	Light, Microscope, Drying oven	Easy	Low	Less time consuming, high productivity, wide angle ageing	Difficulty counting growth bands from older sharks	This study

accuracy of the burn method and other traditional techniques across a range of elasmobranchs is necessary to determine how widely this approach can be applied to other species.

ACKNOWLEDGEMENTS

We greatly appreciate the dedication and help of K. Ishida, T. Oyama, M. Onodera, and S. Onodera for sample collection and measurement recording. We thank the crew of the commercial long-line fleet from Kesennuma fishing port, Japan for their tremendous help sampling across a vast area of the western North Pacific Ocean. We appreciate Dr. M. Sakai, Dr. H. Kidokoro, Dr. S. Suyama, and Dr. M. Nakagami, Tohoku National Fisheries Research Institute, Japan Fisheries Research and Education Agency, and Professor M. Nemoto, Tokyo University of Marine Science and Technology, for kindly assisting with sampling a wide range of specimens. The first author deeply appreciates the dedicated help of A. Matsushita and Y. Hayashi for processing and measuring vertebral samples. The first author also thanks Dr. Y. Hiraoka, Japan Fisheries Research and Education Agency, for her helpful comments on statistical analyses. Finally, we sincerely thank an anonymous reviewer for valuable comments on an earlier draft of this manuscript. This study was supported by the Fisheries Agency, Government of Japan.

ORCID

Y. Fujinami http://orcid.org/0000-0001-7569-3970
S. Ohshimo http://orcid.org/0000-0002-2883-7588

REFERENCES

Andrews, A. H., Natanson, L. J., Kerr, L. A., Burgess, G. H., & Cailliet, G. M. (2011). Bomb radiocarbon and tag-recapture dating of sandbar shark (*Carcharhinus plumbeus*). *Fishery Bulletin*, *109*, 454–465.

Başusta, N., Demirhan, S. A., Çiçek, E., & Başusta, A. (2017). Comparison of staining techniques for age determination of some Chondrichthyan species. *Turkish Journal of Fisheries and Aquatic Sciences*, *17*, 41–49.

Beamish, R. J., & Fournier, D. A. (1981). A method for comparing the precision of a set of age determination. *Canadian Journal of Fisheries and Aquatic Sciences*, *38*, 982–983. https://doi.org/10.1139/f81-132

Blanco-Parra, M. D. P., Galvan-Magnna, F., & Marquez-Farias, F. (2008). Age and growth of the blue shark, *Prionace glauca* Linnaeus, 1758, in the northwest coast of Mexico. *Revista de Biologia Marina y Oceanografia*, *43*, 513–520.

Cailliet, G. M., Martin, L. K., Kusher, D., Wolf, P., & Welden, B. A. (1983). Techniques for enhancing vertebral bands in age estimation of California elasmobranchs. In: E. D. Prince & L. M. Pulos (Eds.), *Proceedings of the international workshop on age determination of oceanic pelagic fishes: Tunas, Billfishes, Sharks, 15–18 February 1982, Miami, FL, USA* (pp. 157–165), NOAA Technical Report NMFS 8.

Campana, S. E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age

validation methods. *Journal of Fish Biology*, *59*, 197–242. https://doi.org/10.1111/j.1095-8649.2001.tb00127.x

Campana, S. E. (2014). *Age determination of elasmobranchs, with special reference to Mediterranean species: A technical manual*. Rome, Italy: FAO.

Campana, S. E., Annand, M. C., & McMillan, J. I. (1995). Graphical and statistical methods for determining the consistency of age determination. *Transactions of the American Fisheries Society*, *124*, 131–138. https://doi.org/10.1577/1548-8659(1995)124<0131:GASMFD>2.3.CO;2

Carlson, J. K., & Baremore, I. E. (2003). Changes in biological parameters of Atlantic sharpnose shark *Rhizoprionodon terraenovae* in the Gulf of Mexico: Evidence for density-dependent growth and maturity? *Marine and Freshwater Research*, *54*, 227–234. https://doi.org/10.1071/MF02153

Casey, J. G., Pratt, H. L. Jr, & Stillwell, C. (1985). Age and growth of the sandbar shark (*Carcharhinus plumbeus*) from the western North Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences*, *42*, 963–975. https://doi.org/10.1139/f85-121

Chang, W. Y. B. (1982). A statistical method for evaluating the reproducibility of age determination. *Canadian Journal of Fisheries and Aquatic Sciences*, *39*, 1208–1210. https://doi.org/10.1139/f82-158

Christensen, J. M. (1964). Burning of otoliths, a technique for age determination of soles and other fish. *ICES Journal of Marine Science*, *29*, 73–81. https://doi.org/10.1093/icesjms/29.1.73

Francis, M. P., & Maolagáin, C. Ó. (2000). Age, growth and maturity of a New Zealand endemic shark (*Mustelus lenticulatus*) estimated from vertebral bands. *Marine and Freshwater Research*, *51*, 35–42. https://doi.org/10.1071/MF99012

Fujinami, Y., Semba, Y., Okamoto, H., Ohshimo, S., & Tanaka, S. (2017). Reproductive biology of the blue shark, *Prionace glauca*, in the western North Pacific Ocean. *Marine and Freshwater Research*, *68*, 2018–2027. https://doi.org/10.1071/MF16101

Geraghty, P. T., Jones, A. S., Stewart, J., & Macveth, W. G. (2012). Micro-computed tomography: An alternative method for shark ageing. *Journal of Fish Biology*, *80*, 1292–1299. https://doi.org/10.1111/j.1095-8649.2011.03188.x

Goldman, K. J., Cailliet, G. M., Andrews, A. H., & Natanson, L. J. (2012). Assessing the age and growth of chondrichthyan fishes. In J. C. Carrier, J. A. Musick, & M. R. Heithaus (Eds.), *Biology of sharks and their relatives* (2nd ed., pp. 423–451). Boca Raton, FL: CRC Press. https://doi.org/10.1201/CRCMARINEBIO

Hale, L. F., Dudgeon, J. V., Mason, A. Z., & Lowe, C. G. (2006). Elemental signatures in the vertebral cartilage of the round stingray, *Urobatis halleri*, from Seal Beach, California. *Environmental Biology of Fishes*, *77*, 317–325. https://doi.org/10.1007/s10641-006-9124-2

Harry, A. V. (2018). Evidence for systematic age underestimation in shark and ray ageing studies. *Fish and Fisheries*. https://doi.org/10.1111/faf.12243

Hoening, J. M., & Brown, C. A. (1988). A simple technique for staining growth bands in elasmobranch vertebrae. *Bulletin of Marine Science*, *42*, 334–337.

ISC. (2012). Report of the 12th meeting of the International Scientific Committee for Tuna and Tuna-like Species in the North Pacific Ocean, Annex-9, 18–23 July 2012, Sapporo, Hokkaido, Japan.

ISC. (2014). Stock assessment and future projections of blue shark in the North Pacific Ocean. Report of the 14th meeting of the International Scientific Committee for Tuna and Tuna-like Species in the North Pacific Ocean plenary session, Annex-13, 16–21 July 2014, Taipei, Chinese-Taipei.

Jolly, K. A., da Silva, C., & Attwood, C. G. (2013). Age, growth and reproductive biology of the blue shark *Prionace glauca* in South African waters. *African Journal of Marine Science*, *35*, 99–109. https://doi.org/10.2989/1814232X.2013.783233

Joung, S. J., Lyu, G. T., Su, K. Y., Hsu, H. H., & Liu, K. M. (2017). Distribution pattern, age, and growth of blue sharks in the South Atlantic Ocean. *Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science*, *9*, 38–49. https://doi.org/10.1080/19425120.2016.1249579

Manning, M. J., & Francis, M. P. (2005). Age and growth of blue shark (*Prionace glauca*) from the New Zealand Exclusive Economic Zone. New Zealand Fisheries Assessment Report, 2005/26. Ministry of Fisheries, Wellington, New Zealand.

Matta, M. E., Tribuzio, C. A., Ebert, D. A., Goldman, K. J., & Gburski, C. M. (2017). Age and growth of elasmobranchs and applications to fisheries management and conservation in the Northeast Pacific Ocean. In S. E. Larson & D. Lowry (Eds.), *Advances in marine biology* (Vol. *77*, pp. 179–220). Oxford: Academic Press.

Nakano, H. (1994). Age, reproduction and migration of blue shark in the North Pacific Ocean. *Bulletin of National Research Institute of Far Seas Fisheries*, *31*, 141–256.

Nakano, H., & Stevens, J. D. (2008). The biology and ecology of the blue shark, *Prionace glauca*. In M. D. Camhi, E. K. Pikitch, & E. A. Babcock (Eds.), *Sharks of the open ocean: Biology, fisheries and conservation* (pp. 140–151). Oxford: Blackwell Publishing Ltd. https://doi.org/10.1002/9781444302516

Natanson, L. J., & Cailliet, G. M. (1990). Vertebral growth zone deposition in Pacific angel sharks. *Copeia*, *1990*, 1133–1145. https://doi.org/10.2307/1446499

Natanson, L. J., & Campana, S. E. (2002). Validated age and growth of the porbeagle shark (*Lamna nasus*) in the western North Atlantic Ocean. *Fishery Bulletin*, *100*, 266–278.

Ohshimo, S., Shiraishi, T., Tanaka, H., Yasuda, T., Yoda, M., Ishida, H., & Tomiyasu, S. (2014). Growth and reproductive characteristics of the roughear scad *Decapterus tabl* in the East China Sea. *Japan Agricultural Research Quarterly*, *48*, 245–252. https://doi.org/10.6090/jarq.48.245

Oshitani, S., Nakano, H., & Tanaka, S. (2003). Age and growth of the silky shark *Carcharhinus falciformis* from the Pacific Ocean. *Fisheries Science*, *69*, 456–464. https://doi.org/10.1046/j.1444-2906.2003.00645.x

R Development Core Team. (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/.

Raoult, V. R., Peddemors, V. M., Zahra, D., Howell, N., Howard, D. L., De Jounge, M. D., & Williamson, J. E. (2016). Strontium mineralization of shark vertebrae. *Scientific Reports*, *6*, 29698. https://doi.org/10.1038/srep29698

Ricker, W. E. (1975). Computational and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada*, *191*, 1–382.

Scharer, R. M., Patterson, W. F. III, Carlson, J. K., & Poulakis, G. R. (2012). Age and growth of endangered smalltooth sawfish (*Pristis pectinate*) verifies with LA-ICP-MS analysis of vertebrae. *PLoS ONE*, *7*, e47850. https://doi.org/10.1371/journal.pone.0047850

Semba, Y., Nakano, H., & Aoki, I. (2009). Age and growth analysis of the shortfin mako, *Isurus oxyrinchus*, in the western and central North Pacific Ocean. *Environmental Biology of Fishes*, *84*, 377–391. https://doi.org/10.1007/s10641-009-9447-x

Skomal, G. B., & Natanson, L. J. (2003). Age and growth of the blue shark (*Prionace glauca*) in the North Atlantic Ocean. *Fishery Bulletin*, *101*, 627–639.

Sminkey, T. R., & Musick, J. A. (1995). Age and growth of the sandbar shark, *Carcharhinus plumbeus*, before and after population depletion. *Copeia*, *1995*, 871–885. https://doi.org/10.2307/1447035

Stevens, J. D. (1975). Vertebral rings as a means of age determination in the blue shark (*Prionace glauca* L.). *Journal of the Marine Biological Association of the United Kingdom*, *55*, 657–665. https://doi.org/10.1017/S0025315400017318

Tanaka, S., Cailliet, G. M., & Yudin, K. G. (1990). Differences in growth of the blue shark, *Prionace glauca*: Technique or population? In H. L. Pratt, S. H. Gruber & T. Taniuchi (Eds.), *Elasmobranchs as living resources: Advances in the biology, ecology, systematics, and status of the fisheries*(pp. 177–187). NOAA Technical Report No. 90.

Tanaka, S., Kitamura, T., Mochizuki, T., & Kofuji, K. (2011). Age, growth and genetic status of the white shark (*Carcharodon carcharias*) from Kashima-nada, Japan. *Marine and Freshwater Research*, *62*, 548–556. https://doi.org/10.1071/MF10130

Wells, R. J. D., Spear, N., & Kohin, S. (2017). Age validation of the blue shark (*Prionace glauca*) in the eastern Pacific Ocean. *Marine and Freshwater Research*, *68*, 1130–1136. https://doi.org/10.1071/MF16054

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Fujinami Y, Semba Y, Ohshimo S, Tanaka S. Development of an alternative ageing technique for blue shark (*Prionace glauca*) using the vertebra. *J Appl Ichthyol*. 2018;34:590–600. https://doi.org/10.1111/jai.13620