Techniques for Enhancing Vertebral Bands in Age Estimation of California Elasmobranchs

GREGOR M. CAILLIET, LINDA K. MARTIN, DAVID KUSHER, PATRICIA WOLF, and BRUCE A. WELDEN[1]

ABSTRACT

Vertebrae from 1,152 elasmobranchs representing 22 species were collected between 1979 and 1981 to assess methods of enhancing incremental growth bands for age estimation. Thus far, we have tested methods previously reported in the literature, and have developed new procedures to enhance growth increments on 684 individuals of 14 species of elasmobranchs. Silver nitrate impregnation, X-radiography, and cedarwood oil clearing were the most successful techniques. Less effective were alizarin red staining, paraffin impregnation, alcohol immersion, and formic acid etching. Methods for preparing vertebrae and enhancing and counting growth increments are presented, and the problems associated with interpreting the annual nature of such counts are discussed.

INTRODUCTION

Little is known about the age, growth, and reproduction of elasmobranchs because many species are difficult to sample, are of relatively large size, are highly mobile, exhibit seasonality, and are of minor commercial value. In addition, many of the conventional age determination methods used for bony fishes are not applicable to elasmobranchs because elasmobranchs lack calcareous otoliths and other skeletal hardparts. In California, the commercial exploitation of elasmobranchs has been rapidly increasing, making information about their life histories essential for understanding and managing their populations.

Several methods of age determination have been developed for elasmobranchs. Length frequency analysis has been used by Templeman (1944), Olsen (1954), Aasen (1963), Parker and Stott (1965), Johnson and Horton (1972), Sage et al. (1972), and Edwards (1980). Often, this kind of analysis is coupled with tag-recapture studies (Steven 1936; Kauffman 1955; Babel 1967; Davies and Joubert 1967; Kato and Carvallo 1967; Wass 1973; Holden 1974; and Grant et al. 1979). These two approaches are limited due to the slow growth rates exhibited by elasmobranchs, and sampling difficulties. Moss (1972) used tooth replacement rates to estimate growth rates, but this technique provides only rough estimates, as the tooth replacement rate varies among individuals. Using the developmental state of secondary sex characters, Johnson and Horton (1972) could only categorize fish into "young, immature, and adult age groups." Embryonic growth rates have also been used to generate growth curves by extrapolation (Ketchen 1972; Holden 1974; Francis 1981), but "it is not a substitute for growth rate analysis based on age determination, and should only be used as an interim measure" (Francis 1981). Dorsal spines have been examined by Kaganovskaia (1933), Templeman (1944), Bonham et al. (1949), Aasen (1961), Holden and Meadows (1962), and Ketchen (1975), and were found to have incremental zones (see Glossary). Because most elasmobranchs do not have spines, this technique has limited applicability.

Growth zones deposited in vertebral centra are promising tools for age determination of elasmobranchs. Ridewood (1921) first described these zones in his review of calcification processes, and Urist (1961) and Applegate (1967) provided further morphological evidence that these zones were common among sharks and rays. Haskell (1949) first suggested that these zones could be useful in age determination studies. Several authors then developed and used various techniques to enhance these zones in several species of elasmobranchs, including alcohol immersion (Richards et al. 1963), xylene impregnation (Daiber 1960), alizarin red (LaMarca 1966), histology (Ishiyama 1951), silver nitrate impregnation (Haskell 1949; Stevens 1975), X-radiography (Urist 1961; Aasen 1963; Applegate 1967), and X-ray spectrometry (Jones and Geen 1977).

Various authors have postulated that these growth zones are deposited annually. Ishiyama (1951), working with the Japanese black skate, *Raja fusca*, tentatively concluded that the alternating zones were laid down in winter. Daiber (1960) and Richards et al. (1963) found that their growth data for two other species of skate fit the von Bertalanffy (1938) growth equation, and concluded that their zones were probably annual. Stevens (1975) estimated age of blue sharks, *Prionace glauca*, using silver nitrate, and found that his data correlated well with Aasen's (1966) length-frequency data. Several authors have used tetracycline to mark bony structures in fishes (Weber and Ridgway 1962; Simkiss 1974), and recent studies using tetracycline on elasmobranchs (Holden and Vince 1973; Graber and Stout 1983) support annual zone formation in their centra. Finally Jones and Geen (1977) used an energy-dispersive X-ray spectrometric system to detect peaks of the elements calcium and phosphorus, which they concluded were deposited annually in the centra of the spiny dogfish, *Squalus acanthias*.

One of the answers to Holden's (1977) plea for "establishing acceptable techniques" in age determination of elasmobranchs may lie in the concentric zones found in their centra. Because the amount and pattern of calcification may vary considerably among species (Ridewood 1921; Haskell 1949; Urist 1961; LaMarca 1966; Applegate 1967), a comprehensive review and evaluation of age determination methodology are needed. Since 1979, we have attempted to determine the most effective methods of enhancing the visibility of these zones in centra

[1]Moss Landing Marine Laboratories, Moss Landing, CA 95039-0223.

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from California elasmobranchs. We have experimented with cleaning, slicing, and grinding procedures to prepare vertebrae for subsequent age determination, and have used numerous enhancement methods to expose the zones for counting. We present here our evaluation of several enhancement methods, and discuss counting procedures and problems associated with interpreting the periodicity of zone deposition.

COLLECTING, PROCESSING, AND PREPARATION

We have utilized several sources to obtain specimens for testing the various age determination techniques. The catches at several shark derbies conducted in Elkhorn Slough, Calif., (Herald et al. 1960) provided us with samples of the leopard shark, *Triakis semifasciata*, brown smoothhound, *Mustelus henlei*, and bat ray, *Myliobatis californica*. We have also sampled with trawls and gill nets to obtain other local coastal species such as the blue shark, gray smoothhound, *Mustelus californicus*, and spiny dogfish. Commercially important elasmobranchs were obtained in central and southern California by subsampling the commercial gill net, trammel net, and trawl fishing fleet catches. This produced specimens of the common thresher, *Alopias vulpinus*, shortfin mako, *Isurus oxyrinchus*, soupfin, *Galeorhinus zyopterus*, and Pacific angel, *Squatina californica*, sharks, in addition to the longnose, *Raja rhina*, and big, *R. binoculata*, skates. Specimens of the basking shark, *Cetorhinus maximus*, and the great white shark, *Carcharodon carcharias*, were obtained incidental to commercial gill net catches (Table 1). We have collected 1,152 specimens representing 22 species.

For each individual specimen collected, measurements were taken, the reproductive tract examined, and approximately 12 vertebrae removed, usually from below the origin of the dorsal fin, and frozen in plastic bags. Measurements included length (total, precaudal, and distance between dorsal fin origins), girth, and weight. To assess reproductive condition in males, the configuration of the vas deferens and the condition, size, and development of the claspers were noted. For some species, sperm smears were made and microscopically examined to verify presence of mature sperm (Pratt 1979). For females, the number and size of eggs in the ovaries were recorded; the embryos, if present, measured and sexed; oviducal gland and oviduct dimensions recorded; and presence or absence of uterine scars noted. This information was used to determine the size and age at which the different species reach sexual maturity.

Once defrosted, the neural and haemal arches and connective tissue must be removed from each vertebra to expose the centrum surfaces which contain the zones. This was accomplished using one of several techniques, depending upon the species. For Pacific angel, shortfin mako, common thresher, blue, and great white sharks, a 5-min soak in distilled water followed by air drying effectively allowed the connective tissue to be peeled away from the centrum. Soaking in bleach was more effective for removing connective tissue from leopard shark, gray and brown smoothhounds, spiny dogfish, bat ray, big skate, and longnose skate centra. Longer soaking time in bleach was needed for larger centra, and immersion intervals ranged from 5 to 30 min. Finally, the centrum was rinsed well in tap water. Soaking in enzyme detergent solutions and subjecting the centrum to ultrasonic cleaning procedures did not significantly enhance the cleaning that had already resulted from bleach immersion.

TECHNIQUES FOR ENHANCING BANDS IN VERTEBRAL CENTRA

We have found in nearly every elasmobranch centrum examined that the zones are the result of two kinds of concentric marks (Fig. 1). We define a "ring" as the narrowest kind of concentric mark observed, and use the term "band" to refer to wider concentric marks composed of groups of rings. Therefore, we interpret the wider bands to contain widely spaced rings, while narrow bands have rings that are more tightly spaced.

Cleaned centra were often sectioned, either along a transverse or longitudinal plane (Fig. 1b) to prepare them for three band-enhancement techniques. This sectioning was especially

Table 1.—Summary of collection and processing activities from 1979 to 1981 showing the number of each species of elasmobranch collected and aged, and the relative effectiveness of three techniques for clarifying bands in vertebral centra. These techniques were evaluated as those that provided repeatable counts (+), did not provide repeatable counts (−), or were not tried (?).

Common name	Scientific name	Number sampled	Number age estimated	Technique		
				Silver nitrate	X-radiography	Oil clearing
Bat ray	*Myliobatis californica*	191	191	−	+	+
Leopard shark	*Triakis semifasciata*	136	131	+	−	?
Brown smoothhound	*Mustelus henlei*	50	50	+	+	?
Gray smoothhound	*Mustelus californicus*	38	38	+	+	?
Common thresher	*Alopias vulpinus*	57	57	+	+	+
Shortfin mako	*Isurus oxyrinchus*	23	23	+	+	+
Blue shark	*Prionace glauca*	26	26	+	+	+
Pacific angel shark	*Squatina californica*	56	41	+	+	?
Soupfin shark	*Galeorhinus zyopterus*	70	0	+	?	?
Longnose skate	*Raja rhina*	196	35	−	−	+
Big skate	*Raja binoculata*	188	50	−	−	+
Spiny dogfish	*Squalus acanthias*	70	40	−	−	?
Basking shark	*Cetorhinus maximus*	2	1	+	+	+
Great white shark	*Carcharodon carcharias*	9	1	+	+	?
		1,112	684			
8 additional species		40	0			
Total		1,152	684			

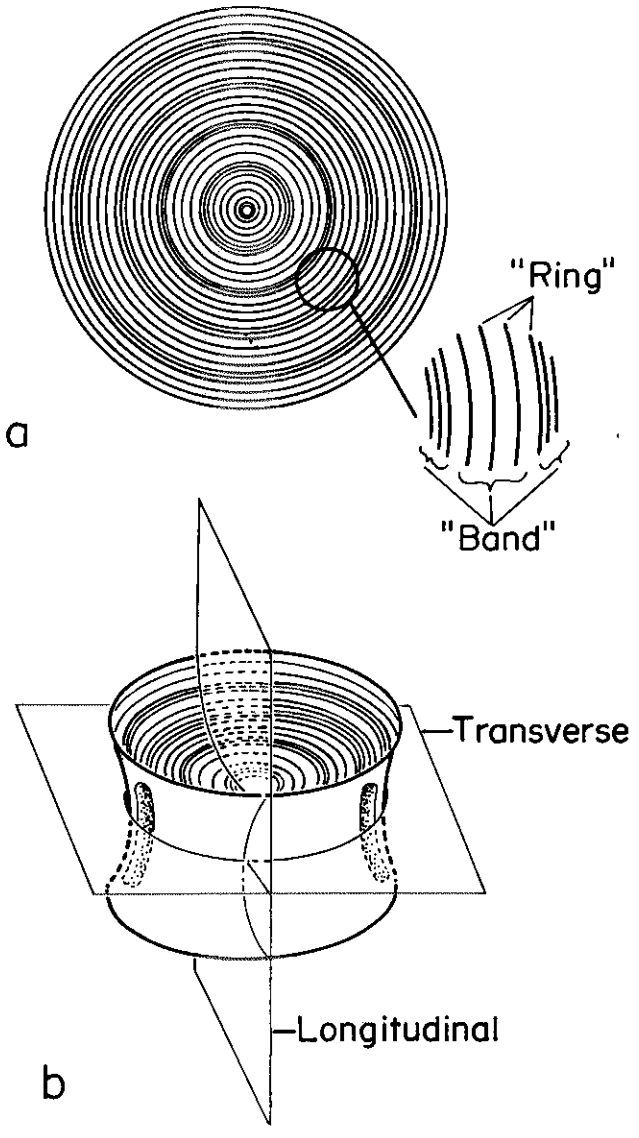

Figure 1.—Diagram of typical elasmobranch centrum showing (a) bands made of fine "rings"; and (b) the two sectioning planes used.

needed for centra that had relatively deep cones (Fig. 2), as opposed to those that were flat or disklike along the longitudinal plane (Figs. 3, 4). Large vertebrae secured in a vise were cut in half with a small circular saw attachment on a jeweler's drill. For smaller specimens, half of the centra was ground away using aluminum oxide wheel points and fine sandpaper attachments for the same tool. Transverse sectioning prevented bands on these opposing halves from obscuring each other when observed after further preparation (Fig. 1), and longitudinal sectioning enhanced the finer bands laid down at the centrum edge.

Three techniques were consistently useful for enhancing bands in centra, while several other techniques have either proven ineffective or have not yet been evaluated.

The first technique was adopted by Stevens (1975) to enhance bands in blue shark centra. Calcium salts in the centrum are replaced with silver, providing distinct silver-impregnated bands which become quite dark after illumination under ultraviolet light. The narrow bands have more tightly spaced rings, and therefore appear darker than the broad bands (Figs. 2, 3a).

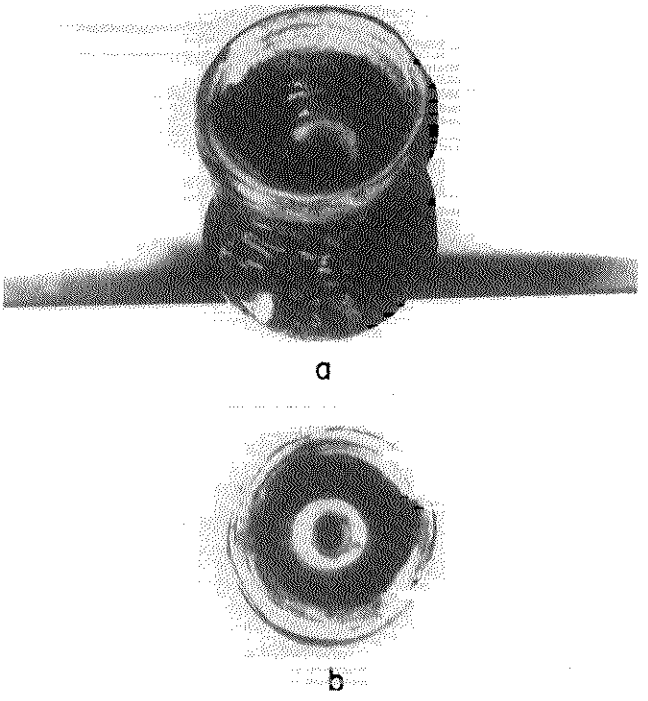

Figure 2. – Oblique (a); and anterior (b) views of the same vertebral centrum taken from a 114 cm TL mature female leopard shark and stained with silver nitrate. This centrum was determined to have nine bands.

An advantage of this technique is that vertebrae preserved in 70% alcohol, as well as fresh specimens, may be used. It was necessary to further modify Stevens' procedures. To assure the chemical substitution of silver for calcium, all connective tissue was removed from the centrum by one of the previous cleaning methods. To remove any traces of bleach and to etch its surface, the centrum was soaked in concentrated (88%) formic acid for 2-4 min. The centrum was then soaked in distilled water for approximately 15 min. Then it was placed in a 1% silver nitrate solution and immediately placed in a chamber where it was illuminated by an ultraviolet light source (GE[2] F15T8-BLB) for 3-15 min, depending upon the species tested and the size of the centrum. The centrum was then rinsed in distilled water to remove excess silver nitrate.

Usually, a dissecting microscope with reflected illumination focused laterally on the centrum was used to count bands. Several centra (3-5) from each specimen were stained and counted for replicate analysis. After these counts were made on the newly stained centra, they were soaked in a 5% sodium thiosulfate solution for 2-3 min. This procedure removes excess silver and fixes the chemical substitution. Because fixation often eradicates the very narrow rings, counts should occur before fixation if counts of these rings are desired. Band counts were made before and after fixation. The final step was storage in 70% isopropyl alcohol.

The second technique involved taking X-radiographs of half-centra as prepared above. We have used a Hewlett-Packard Faxitron Series X-Ray System (Model No. 43805N) with Kodak Industrex M film (Readypack M-2), as suggested by Miller and Tucker (1979). X-radiographs of bat ray centra were viewed

[2]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

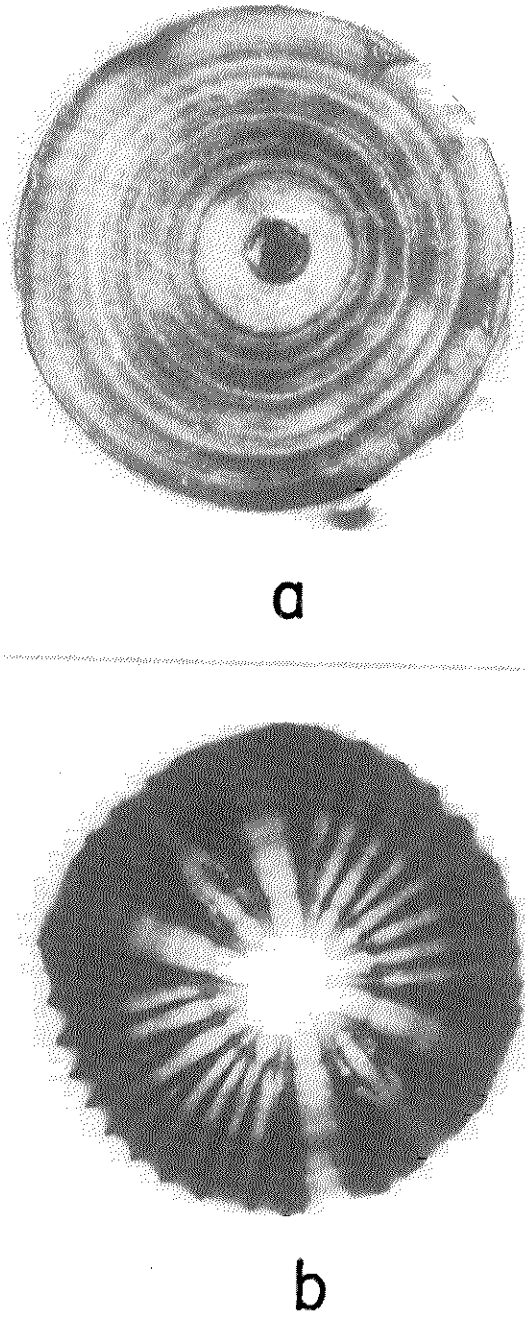

Figure 3.—Two vertebral centra from the same specimen of shortfin mako shark with bands enhanced using (a) the silver nitrate technique; and (b) the X-radiography method. The centrum diameter from this 211 cm TL immature female was 26 mm, and there were six or seven bands, as determined by these two techniques.

through a dissecting microscope with a combination of reflected and transmitted light. X-radiographs of centra from Pacific angel, common thresher, blue, basking, great white, shortfin mako, and both species of smoothhound sharks were viewed through a compound or dissecting microscope using transmitted light from below. In X-radiographs, the narrow bands appear white, while the broad bands, which have less tightly spaced rings, appear darker (Figs. 3b, 4).

The third technique involved applying cedarwood oil to the face of each centrum to increase the clarity of bands by eliminating superficial irregularities. Frequently, scraping of the

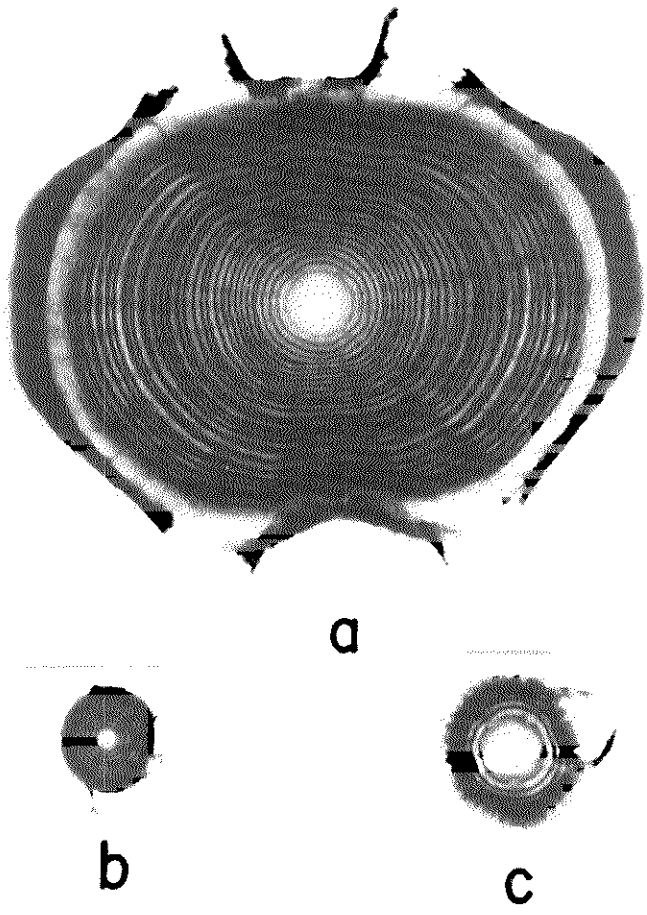

Figure 4.—Radiographs of the vertebral centra of three sizes and developmental stages of Pacific angel shark. (a) is from a 1,110 mm TL adult female, widest centrum diameter 17 mm; (b) is from a 360 mm TL free-living juvenile, centrum diameter 6 mm; (c) is from an unborn, pre-term embryo with yolk sac that measured 225 mm TL and had a 3 mm centrum diameter.

centrum face with a scalpel enhanced the clarity of the finer bands. The bands of the centrum thus prepared were viewed under a dissecting microscope using a fiber optics light transmitted both vertically and horizontally over a dark background. Using this approach, the bands that are composed of more tightly spaced rings (narrow bands) appear darker than those with less tightly spaced rings (broad bands).

Attempts at using several other published techniques were less successful at enhancing bands in centra for the species we examined. Following Daiber's (1960) technique for the clearnose skate, *Raja eglanteria*, centra from both the longnose and big skates were soaked in Formalin, cleared of connective tissue, and placed in 95% isopropyl and then in absolute isopropyl alcohol. After immersion in xylene, the centra were heated in paraffin at 60 °C and returned to xylene. Most of the centra prepared in this manner were only partially cleared, and bands were unclear. In testing the technique of Richards et al. (1963), centra from the same two skate species were either cleaned in sodium hydroxide or scraped, placed first in 70% isopropyl alcohol and then transferred to 100% alcohol. Bands especially on the outer portions of the centrum, were unclear, irrespective of cleaning technique. Using a third method, originally used by LaMarca (1966) on the sand tiger shark, *Odontaspis taurus*, a small number of centra from longnose skates were

cleared in sodium hydroxide, stained in a saturated solution of alizarin red S in sodium hydroxide, and differentiated in 3% hydrogen peroxide. Although success in enhancing growth bands on centra was variable and ring contrast was moderate, further attempts with this technique are warranted and may yield better results. Smith (1980) used a technique on the cownose ray, *Rhinoptera bonasus*, in which vertebrae were stored in alcohol, cleaned, air-dried, and sectioned longitudinally. After the hourglass-shaped face was polished, the centra were heated at 200°C for 2-3 min. This technique did not enhance bands in centra of the Pacific bat ray. Finally, our attempt to use Stirling's (1969) method originally designed for delineating rings in pinniped teeth, which involves etching for 24 h with formic acid and Formalin, did not noticeably enhance bands in leopard shark centra.

Undoubtedly, there are many other procedures that may prove useful in aiding researchers to enhance bands in elasmobranch vertebrae. One, which we have not yet had the facilities to pursue, is the use of X-ray or electron microprobe spectrometry to measure such elements as calcium and phosphorus, which are more concentrated during certain seasons than in others (Jones and Geen 1977; Casselman 1983). This method is somewhat expensive and time-consuming, but may provide valuable information for comparing results with other, more practical techniques.

EFFECTIVENESS OF TECHNIQUES

Procedures for counting bands in centra were standardized to ensure consistent and objective evaluation of the various techniques among species and among researchers. For all species and techniques, at least two observers made replicate and independent counts of both narrow and broad bands in each centrum. If these initial counts varied by more than one set of bands and additional readings did not result in agreement, the centrum was not used for age analysis. In general, there was good agreement among observers, with $< 10\%$ of the counts disagreeing by more than one band pair. As is common in many age determination studies, there was greater agreement of band counts from younger age classes. Each technique was considered to be effective if it followed these criteria and produced consistent and repeatable band counts.

We were able to delineate and count bands on centra of all species tested using at least one of the three enhancement techniques (Table 1). Using our modification of the Stevens' (1975) silver nitrate staining technique, bands were clearly discernible in 10 of the 14 species tested, and examples of centra treated with silver nitrate are shown in Figures 2 and 3b. This technique did not produce repeatable band counts in species that had centra with a poorly differentiated calcification pattern, poor calcification, or only narrow and tightly spaced bands. Schwartz (1983) also used this method to estimate age of dusky, *Carcharhinus obscurus*, and scalloped hammerhead, *Sphyrna lewini*, sharks off North Carolina.

Distinct bands were discernible in 9 of the 13 species tested using X-radiography (Table 1; Figs. 3b, 4). The X-ray technique was not successful in providing repeatable counts with centra of the spiny dogfish, leopard shark, and the two skates tested, apparently due to diffuse calcification patterns, and to radiating structural components in these vertebrae that interfere with the clarity of the bands (Ridewood 1921). For species with narrow, elongate (deep-coned) centra, such as gray and brown smoothhounds, it was necessary to section these centra longitudinally so that the bands could be clearly observed in radiographs.

The oil-clearing technique worked well on all seven species tested (Table 1). Because transmitted light was used with this technique, it detected differences in optical density through the centrum, and was not simply an examination of surface topography.

In general, those specimens with ages estimated by any two of these techniques (Table 1) agreed with each other. For example, 92% of the 130 bat rays aged by both X-radiography and oil clearing were placed in the same age class or differed by only 1 yr, with most of the disagreements occurring in the oldest fish (Martin 1982). Similar results for smaller samples were found for the common thresher, shortfin mako, blue, and basking sharks. In species in which band counts were compared using both X-radiography and silver nitrate, counts were also extremely similar. For example, 90% of the 31 gray and 82% of the 45 brown smoothhounds aged by these two techniques were placed in the same age class or differed by only 1 yr (Kusher[3]). Centra prepared by these two techniques for the shortfin mako also produced quite similar results, with the example shown in Figure 3 producing counts of 6 or 7 bands for the same individual. Similar results from smaller samples were found for the common thresher, shortfin mako, blue, Pacific angel, basking, and great white sharks.

INTERPRETATION OF BAND COUNTS

Once a sufficient number of centra has been used to estimate age by counting bands, growth curves can be constructed using several models that have been reviewed extensively by Ricker (1979). To confidently interpret the meaning of band counts in elasmobranch centra, it must be demonstrated that band formation provides a continuous record of growth, and the count of bands represents known intervals of time.

The assumption that centra are good indicators of age is supported by three lines of evidence. First, in elasmobranchs, growth of the calcified cartilagenous skeleton occurs by a one-way process of deposition, and there is no indication of internal remodeling or resorption (Ridewood 1921; Urist 1961; Applegate 1967; Simkiss 1974). Second, increased body sizes are accompanied by increases in centrum diameters (Stevens 1975), because the centrum must grow in order to accommodate new growth bands. Third, because the banding pattern visible in X-rays (Gosline 1948) and in the other two techniques occurs as a result of density differences in subject matter, it is likely that the difference between the high and low density bands is due to differences in mineralization occurring during different growth phases. As suggested by Ishiyama (1951) and Jones and Geen (1977), the pattern of mineralization may be strongly influenced by seasonal environmental changes which may, in turn, affect growth rates. The presence of a heavily mineralized peripheral band in the majority of young bat rays and leopard sharks collected during the summer months, and a lightly mineralized band in winter-caught specimens, offer further sup-

[3]Kusher, D. Age, growth and reproduction of the leopard shark, *Triakis semifasciata*, gray smoothhound, *Mustelus californicus*, and brown smoothhound, *Mustelus henlei*. M.A. Thesis in prep. Moss Landing Marine Laboratories, P.O. Box 223, Moss Landing, CA 95039.

port for the assumption of faster growth in summer and slower growth in winter (Martin 1982; Kusher footnote 3).

It is generally accepted, but still an assumption, that the growth bands found in the hardparts of temperate teleosts are of an annual nature (Williams and Bedford 1974; Holden 1977), but this has not been adequately validated for bands in elasmobranch centra. Numerous authors studying elasmobranch growth have postulated or assumed annual band formation in centra (Aasen 1963; Richards et al. 1963; Taylor and Holden 1964; Stevens 1975), but conclusive age validation studies using such techniques as tag-recapture and tetracycline injection have only been tested on several elasmobranch species (Holden and Vince 1973; Holden 1974; Gruber and Stout 1983).

For several Pacific species, we have produced growth curves and have used several approaches to assess whether their bands are annual. Often, different verification methods produce inconclusive or conflicting results, and seldom can the majority of them be applied to one species (Brothers 1983). These methods can be characterized as those which: 1) Require random sampling of numerous individuals over time while monitoring changes in their size classes and centrum characteristics, 2) compare vital growth model parameters with known size information, such as size at birth (L_0) and maximum observed size (L_∞), and 3) measure individual growth using tagged fish from field recaptures or in laboratory growth experiments. This last approach can also include centrum band-marking techniques.

The first verification approach is one of the most commonly used, because some species are relatively easy to sample. We have compiled size-frequency histograms for bat rays, leopard sharks, gray and brown smoothhounds, and blue sharks, and have compared the mean sizes of the first several modes with the growth increments predicted by growth curves generated from band counts with good agreement (Kusher footnote 3). Also, the changes in mean size of young-of-the-year leopard sharks collected monthly in Elkhorn Slough corresponded well with early growth as determined from band counts, thus verifying that band counts can be used as indicators of early ages (Kusher footnote 3).

In newborn individuals, the number of bands can be used as an indication of gestation period, assuming the bands are laid down over some regular interval prior to birth. Newborn leopard sharks lack a complete set of bands, one dark and the other lighter, using silver nitrate impregnation, suggesting that these band pairs may be laid down at least annually, and, if so, that their gestation period is a year or less (Kusher footnote 3). Because leopard sharks in central California are born in spring and early summer, and the center of their centrum is dark when using silver nitrate stain, it is presumed that the dark band represents summer growth, while the lighter band was formed during winter months. Angel shark embryos, on the other hand, have fewer than five bands (Fig. 4c), and newborn individuals have between 6 and 7 bands (Fig. 4) when viewed through X-radiographs, indicating that these bands are laid down in less than annual fashion, mark physiological events, or that these fish have a very long gestation period. Ridewood (1921) depicted a similar number of bands in the vertebra of a ripe embryo of the angel shark, *Squatina squatina*, thus indicating that band formation may be similar among all species in this genus.

The width and density of the centrum edge can also be used to indicate the temporal periodicity of band formation. Because it is difficult without histological preparation to delineate the centrum edge in detail, and because the edge is often irregular in width, we have not yet used the width of the peripheral band to evaluate this approach in elasmobranchs we have studied. However, by categorizing peripheral bands as dark or light when treated with silver nitrate, and comparing the proportion of both summer- and winter-caught specimens with each of these categories of peripheral bands, we have been successful at interpreting seasonality of band formation. For example, most bat ray and leopard shark centra collected in Elkhorn Slough during summer months had dark peripheral bands (Martin 1982; Kusher footnote 3), thus providing indirect evidence that their bands are formed during the summer. This is also supported by the prevalence, during winter months, of the lighter bands at the edges.

Another example of the first approach is to use histological techniques to identify "growing zones" or "peripheral calcification" areas in centrum sections (Ridewood 1921; Urist 1961; Applegate 1967; Andrew and Hickman 1974) in younger individuals collected over time. So far, we have only experimented with this approach, using vertebral centra from a blue shark collected during the summer of 1982. Longitudinal sections 15 μm thick were made using a microtome on centra decalcified with dilute (4%) nitric acid or Cal-Ex, and stained with haemotoxylin and eosin. The decalcification procedure caused some shrinking, so centra should be preserved in Formalin first if measurements are desired, because this procedure reduces the shrinking. Two cell types were apparent in these bow-tie-shaped sections, especially the outside edge. The peripheral cells were narrow or squamous in appearance, while the more proximal adjacent cells were square or cuboidal. Alternating squamous and cuboidal layers continued toward the center of the centrum, but were less distinct. The blue shark examined produced superficial band pair counts totalling six or seven using silver nitrate staining, which agreed with counts made using histological sections. We feel, therefore, that this approach appears promising, and we hope to apply it to centra of other species of elasmobranchs.

The second approach useful in age verification is to compare growth model parameters with known size information, such as size at birth and maximum observed size. Even though growth models may not perfectly fit a given set of size and age estimate data, and information on size at birth and maximum observed size may not be good estimates of mean values, this approach does produce a rough approximation. We have now used this approach comparing vital parameters of the von Bertalanffy (1938) growth equation to size information from catch records for seven species, and results indicated relatively close agreement. Three of these species (the common thresher, shortfin mako, and blue shark) are reported in Cailliet et al. (1983), while three others (the bat ray, *Myliobatis californica*, and two species of smoothhound, *Mustelus henlei* and *M. californica*) are reported elsewhere (Martin 1982; Kusher footnote 3).

As an example, we report here on the leopard shark, which has an estimated size at birth from many measurements of newborn individuals in central California (200-220 mm TL), which closely corresponds to the length at which the von Bertalanffy curve, based on 130 aged specimens, intersects the ordinate (Kusher footnote 3). In addition, maximum reported size from our catch records and from Miller and Lea (1972) is only 13% higher than the asymptotic length derived for females (Kusher footnote 3).

Although these preliminary results must be tempered by small sample size, and the unsubstantiated assumption that one set of bands is equivalent to 1 yr, our method of counting bands appears to follow the von Bertalanffy growth model for the size range of leopard sharks we examined. Holden (1974) also found that this approach supported estimates of age for several species of skates that he studied.

The third approach, which can be used in both the field and the laboratory, is to monitor the change in body size of tagged individuals over known periods of time. There are problems associated with this approach, but it generates information with which to evaluate growth curves. In the field, difficulties arise in collecting sufficient numbers of animals, making accurate measurements, tagging them without harming them or inhibiting their natural growth rates, and finally, recapturing them after a sufficient period of time has elapsed during which growth can be measured. We have been able to successfully use this approach on leopard sharks tagged by us in Elkhorn Slough, and tagged in San Francisco Bay (Smith[4]). We have plotted lengths and ages (based upon number of bands counted on centra at recapture) on the growth curve and compared them with the size at time of first capture. The slope and position of changes in size have been quite helpful in evaluating our growth curve.

For leopard sharks, several recaptured fish fit the growth curve closely, but several others did not grow, even over 2 yr (Kusher footnote 3). This indicates that all individuals do not grow exactly as the curve would predict or that tagged fish were poorly measured or did not grow. The size and presumed age of tagged individuals will influence this verification technique considerably, because older fish grow more slowly, and changes in their sizes will be less detectable. Thus, growth rates based on tag recapture data from one size class cannot be used to calculate a growth rate for all sizes or age classes.

Organisms maintained under laboratory conditions can be used similarly. A major disadvantage of laboratory grow-out studies is often that the fish are not maintained under natural conditions, and so may exhibit unnatural growth rates. We have attempted to grow bat rays and leopard sharks at Moss Landing Marine Laboratories and Steinhart Aquarium, Golden Gate Park, San Francisco, Calif., but have had only limited success, because many of our specimens failed to eat sufficient quantities of food, and often failed to grow at all. However, given improvements in the ability to maintain and grow marine organisms, this approach will provide valuable information, especially of a short-term nature, when measuring growth rates.

Internal marks, such as tetracycline, can be used in conjunction with traditional tag-recapture techniques to determine the time sequence of band formation (Holden and Vince 1973; Gruber and Stout 1983; Casselman 1983). This approach entails injecting a fish, either in the laboratory or field, with tetracycline, which is deposited into areas of calcification. After a known period of time, the fish is recovered and sacrificed, and its centra examined under ultraviolet light for a band of fluorescence. Holden and Vince (1973), Gruber and Stout (1983), and Smith (footnote 4) have used this method successfully on skates, the lemon shark, *Negaprion brevirostris*, and the leopard shark, respectively. However, our attempts with bat rays have not been very successful. The tetracycline did deposit in the peripheral zone of calcification, but it produced a diffuse band too indistinct to serve as a temporal check.

A very promising validation technique involves using radioactive geochronologies to estimate the relative ages of different bands (Goldberg and Bruland 1974; Turekian and Cochran 1981; Casselman 1983). This technique, which we are still developing, involves analyzing inner and peripheral bands of vertebral centra for naturally occurring radionuclides with relatively short half-lives. The difference in radionuclide activity levels between bands can be used to estimate their ages, because these radionuclides have constant and known decay rates. This technique has been used successfully on rockfish, *Sebastes diploproa*, otoliths (Bennett et al. 1982), and to measure growth rates of clams (Turekian et al. 1975, 1979; Turekian and Cochran 1981) and corals (Moore and Krishnaswami 1972; Moore et al. 1973; Dodge and Thompson 1974). Nuclides such as ^{210}Pb (22-yr half-life) and ^{210}Po (138-d half-life) are appropriate for ageing organisms with lifespans up to 100 yr.

Our preliminary analysis of inner and peripheral bands of centra from the common thresher shark indicate that this approach will be successful. The inner band contained 0.04694 ± 0.00420 dpm (disintegrations per minute)/g of ^{210}Po, and the outer band had 0.1082 ± 0.003314 dpm/g (Welden[5]), indicating that sufficient radioactivity and a closed system exist. Thus, provided that ^{210}Pb is present at comparable levels, the time between band formation can be calculated based on the observed ^{210}Po/^{210}Pb ratio.

Elasmobranch age determination requires the use of several validation and verification techniques because of the huge diversity of elasmobranch life histories. For example, methods (applicable to small bottom-dwelling forms) such as tagging, tetracycline marking, and laboratory grow-out studies will be difficult or impossible to apply to large pelagic species (Brothers 1983). A multiple approach is also valuable because the results of several different techniques can be compared for one species. Only with this kind of comprehensive approach will it be possible to confidently state that the bands we have counted provide valid estimates of the age of elasmobranchs.

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[4]S. Smith, Fishery Biologist, Southwest Fisheries Center Tiburon Laboratory, National Marine Fisheries Service, NOAA, 3150 Paradise Drive, Tiburon, CA 94920, pers. commun. 1982.

[5]B. A. Welden. Unpubl. data. Moss Landing Marine Laboratories, P.O. Box 223, Moss Landing, CA 95039.

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