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Age Assessment of the Lemon Shark, *Negaprion brevirostris*, Using Tetracycline Validated Vertebral Centra

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Vertebral centra were removed from 55 tag-recaptured lemon sharks, *Negaprion brevirostris*, that had been injected intramuscularly with tetracycline hydrochloride at a dosage level of 12.5 mg/kg at the time of release. Tetracycline deposited at sites of active calcification on the vertebral centra served as a fluorescent marker along the periphery of each centrum at the time of injection. Thin growth zones, or circuli, were observed in ground and stained sections. A lunar periodicity of approx. 29 d was validated for circulus deposition. Precision of circulus counts was evaluated, with an index of average percent error of 3.4% for a single reader. Age estimates were then obtained using circulus counts for a total of 110 sharks, with a plot of precaudal length against estimated age for both sexes fitting a von Bertalanffy growth curve with the parameters L_∞ = 317.65, k = 0.057, and t_0 = −2.302. The predicted age at maturity for males is 11.6 yr and for females is 12.7 yr. These results show that this species is slow growing and long-lived.

RECENT increased exploitation of elasmobranchs has stimulated research for providing an accurate means of estimating ages in these difficult-to-age fish (Holden, 1974, 1977). Cailliet et al. (1986), in a review of elasmobranch age assessment methods, indicated that the most promising approach involves analysis of growth bands in the vertebral centra. Narrow growth zones form within each major band (Cailliet et al., 1983), but these circuli have usually been regarded as checks (the results of life history events not necessarily related to time passage) and were not considered in the age analyses (Casey et al., 1985). We present evidence that these circuli can be used for age estimation in lemon sharks, *Negaprion brevirostris.*

The temporal periodicity of growth zone deposition must be established for age assessment (Beamish and MacFarlane, 1983). The degree and pattern of calcification varies greatly among species (Urist, 1961; Applegate, 1967; Natanson et al., 1984), therefore each species needs separate investigation. Procedures for evaluating growth zone periodicity have been outlined (Casselman, 1983; Jearld, 1983) and include the important concepts of validation (confirmation of the temporal correspondence of a growth zone) and verification (evaluation of the precision/reproducibility of age estimates/growth zone counts) have been distinguished (Wilson et al., 1983).

Tetracycline (TC) is the only direct method currently used to validate the rate of growth zone formation in wild elasmobranchs. It marks the centra, is deposited at sites of active calcification, and remains distinct for some time. Ages of the European thornback ray, *Raja clavata* (Holden and Vince, 1973), the lemon shark (Gruber and Stout, 1983), the leopard shark, *Triakis semifasciata* (Smith, 1984), and the spiny dogfish, *Squalus acanthias* (Beamish and McFarlane, 1985) have been validated using TC.

Unlike previous elasmobranch ageing work involving the enumeration of annual bands, age and growth parameters of the lemon shark were estimated in this study using TC labeled vertebral centra in conjunction with an improved technique to optimally visualize sub-annual circuli. The temporal periodicity of circulus formation was selected for validation and verification because it occurs more frequently than band deposition and thereby provides a potentially more precise and detailed method of age assessment for this species.

Materials and Methods

During the course of a multiple mark, multiple census tagging program conducted from 1979–86 in the Florida Keys and in Bimini Islands, Bahamas (Gruber, 1982; Henningsen and Gruber, unpubl.), more than 2300 lemon sharks were measured, tagged, injected with TC hydrochloride at a level of 12.5 mg/kg, and released. Precaudal length (PCL) was measured

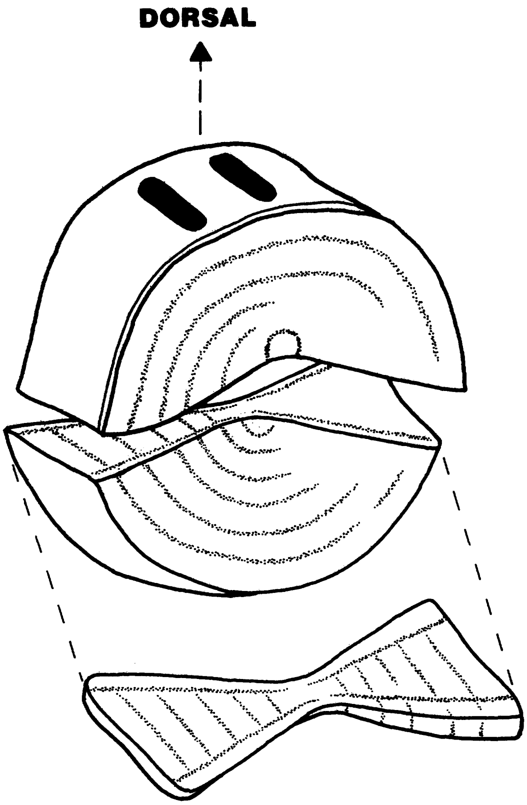

Fig. 1. Plane of sectioning through the centrum, resulting in a bow tie section.

on a straight line from the tip of the snout to the anterior edge of the precaudal pit. Fifty-five recaptured sharks (22 male, 33 female), 46–144 cm PCL and at large from 93–1510 d, were either killed or reinjected with TC and maintained in captivity until death. Additional specimens were rejected if time at large was less than 3 mo or if growth was negligible. These specimens were rejected because centrum growth in lemon sharks is directly related to changes in body length (Gruber and Stout, 1983), and we have observed that circuli are either not deposited or are laid down too closely to each other to be distinguished when the animal does not grow.

To ensure uniformity, the 43rd–47th centra were chosen for processing whenever possible. Although preparation and reading difficulties increased as smaller centra were selected near either end of the vertebral column, circulus counts did not vary substantially among cervical, thoracic, or caudal centra. Frontal sections were cut or ground from centra, using a hacksaw in larger specimens. This resulted in a bow tie shaped face (Fig. 1). Rough sections were polished in water on successively finer grits (180–600) of wet-dry sandpaper. The smooth face was then affixed using cyanoacrylic adhesive to a 50 mm diameter tin disc and each section was further ground to a thickness of 150 μm. This was achieved without decalcification or embedding, thereby preventing the loss of the TC marker.

After removing the adhesive with acetone and transferring to distilled water, the section was stained following a procedure similar to that used by La Marca (1966) on dried centra. One triangular half of each section was immersed for 15 min in a solution made by dissolving 1 g of alizarin red S in 95 ml of distilled water and slowly adding 5 ml of 10% ammonium hydroxide as a buffer. After a thorough rinse in tap water, the half-sections were placed in 20 volume H_2O_2 for 2–4 h to differentiate the stain. This was followed by a distilled water rinse and 3 min of counterstaining in a 1% aqueous solution of Light Green Stain. The other half of each section was left unstained to avoid obscuring the TC marker. All sections were then dehyhdrated through alcohol to xylene and mounted on glass slides using Flotexx mounting medium and glass cover slips, which permit passage of ultraviolet light.

Sections were examined with transmitted light under a compound microscope at 40× magnification, utilizing reflected long-wave ultraviolet light (365 nm) to illuminate the TC marker. Unstained half-sections, on which the circuli were visible, were compared to adjacently mounted and stained half-sections to ensure a complete count. The number of circuli laid down between TC injection and death in the recaptured sharks (or between injections for those sharks which were reinjected and maintained in captivity for some time) was plotted against days at large and linear regression analysis was used to evaluate this relationship. For sharks killed at recapture, the value for days-at-large was adjusted by subtracting 28 d, because the TC marker was not incorporated until 21–35 d after injection (Gruber and Stout, 1983). The slope of the fitted line provided an estimate of the periodicity of deposition.

Circulus counts were obtained from sharks of all sizes, both the 55 recaptured and an additional 55 (29 male, 25 female, 1 sex unknown) first capture sharks ranging up to 226 cm PCL.

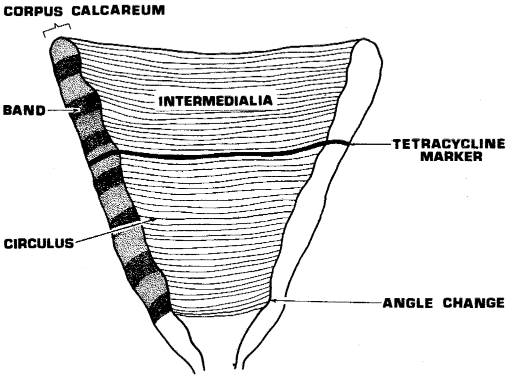

Fig. 2a. Diagrammatic representation of one-half of a 150 μm thick bow tie section.

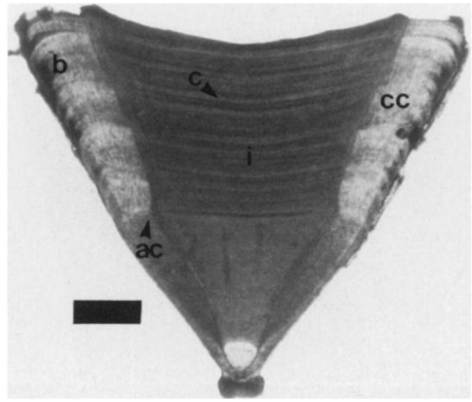

Fig. 2b. Alizarin red S stained section obtained from lemon shark #162, a 90 cm PCL male: cc) corpus calcareum; i) intermedialia; b) band; c) circulus; ac) angle change. Scale = 1 mm.

Count precision was determined by making three nonconsecutive counts using the same reader without previous knowledge of the identity, size or earlier counts for each shark. Each count was the average of at least three consecutive readings. We determined the precision of the age estimates using the method described by Beamish and Fournier (1981) for calculating average percentage error (APE):

$$100 \cdot \frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_j|}{X_j}$$

where

X_{ij} = the i^{th} count for the j^{th} fish,
X_j = the average count for the j^{th} fish and
R = the number of counts for each fish.

This method takes into account the longevity of the species being studied and provides estimates of APE for individual fish. The index of average percent error

$$100 \cdot \frac{1}{N} \sum_{j=1}^{N} \left[\frac{1}{R} \sum_{i=1}^{R} \frac{X|_{ij} - X_j|}{X_j} \right]$$

where

N = the total number of fish aged,

was calculated for samples using a single reader.

The average circulus count for each shark was converted directly into an age estimate using the validated periodicity and was plotted versus size to produce a growth curve for each sex and for the sample population as a whole. Finally, a von Bertalanffy curve was fit to the data points with the aid of a computer program utilizing Marquardt's (1963) algorithm for least squares estimation of nonlinear parameters.

Results

The major features in a ground, 150 μm thick shark vertebral section include the corpus calcareum and the intermedialia (Fig. 2a–b). Circuli were found in the intermedialia and the bands, although present there as well, were usually seen more clearly in the more calcified corpus calcareum. The change in the angle of the corpus calcareum (Fig. 2a–b) is not laid down at birth, but occurs during the first summer. Recaptures that were suspected neonates when first injected (as determined by size, condition, and time of the year) showed that two circuli had been deposited after the TC marker and were proximal to the angle change. Thus, we considered the second circulus proximal to the angle change to be the birth mark. The occasionally visible but faint circuli within that radius were therefore prenatal. Both stained sections, seen with transmitted light, and corresponding unstained sections seen under near-ultraviolet illumination were used to note how many circuli were deposited after the TC marker (Fig. 3).

Circulus deposition had a significant linear relationship to time (Fig. 4). The slope of this line (29.2) provided an estimate of circulus periodicity in days and we used this value to estimate the age of all 110 sharks for which counts had been obtained. The estimated ages of the recaptured sharks ranged from 0.5–8.3 yr. The

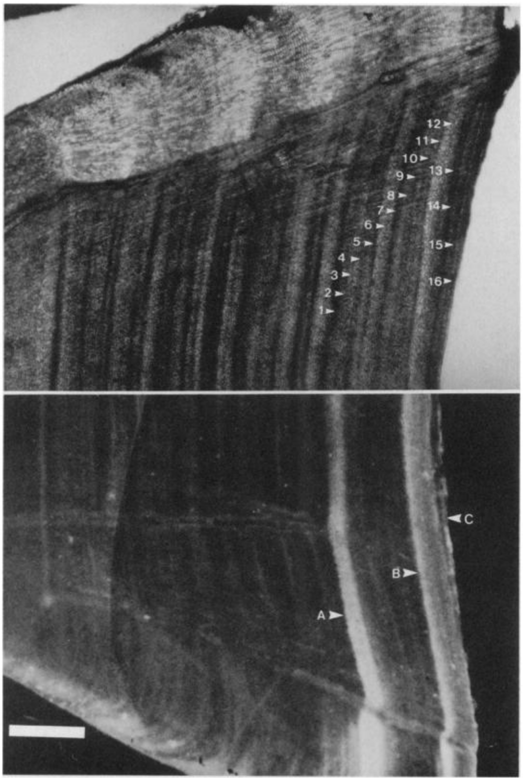

Fig. 3. Composite photograph of stained and unstained halves of bow tie section from shark #162. [LOWER] unstained half viewed in reflected near-UV light: (A) TC marker injected at first capture, 19 June 1982. PCL = 68 cm. Shark released. (B) TC marker injected at second capture, 2 July 1983. PCL = 89 cm. Shark rereleased. (C) Centrum edge existing at third capture, 4 Nov. 1983. PCL = 90 cm. Shark killed. Time between first injection deposition and death (days at large − 28 d deposition period) = 475 d. Scale = 0.5 mm. [UPPER] Stained half exhibiting a total of 51 circuli. Circuli labeled 1–11 were laid down between the first and second TC injections. Circuli labeled 12–16 were deposited after the second injection. Since first injection, one circulus was formed every 29.7 d, on average (16 circuli/475 d).

highest estimated age in this sample was 20.2 yr for a 226 cm PCL male. Some centra were more difficult to read and provided more variable counts than others. Estimates of APE ranged from 0–14.0% for counts on individual sharks. The index of APE was calculated at 3.4% for the sample.

A von Bertalanffy growth equation fitted the combined sex growth curve well (Fig. 5). As the parameters of the growth curve for each sex differed only slightly from those of the combined curve, the separate sex curves were not illustrated. Males and females mature at about 225 and 240 cm in TL (Compagno, 1984), or 175 and 185 cm in PCL, respectively. Using the growth parameters obtained for each sex, the predicted age at maturity for males is about 11.6 yr and for females is about 12.7 yr. Size at birth is predicted to be about 39 cm PCL.

Discussion

The sectioning and staining techniques described here represent a marked improvement over our previous techniques used to visualize sub-annual circuli. Circuli were not sufficiently revealed by staining or etching whole or bisected centra, or through decalcification and thin sectioning. Staining ground sections with silver nitrate results in crystallization which obscures finer circuli. Use of our technique on other species may improve visualization of the similar structures that are present.

The 29 d periodicity of circulus deposition suggests a lunar cycle. Pannella (1971, 1974) has reported both 14 and 28 d recurrent patterns in the otoliths of marine fish. These patterns were always strongly defined in fish that synchronized their activities with the tides. The lunar pattern was found to be superior to annual patterns for ageing tropical species. Campana (1984) found 14–15 d cycles in both increment width and contrast in starry flounder (*Platichthys stellatus*) otoliths and suggested that they were entrained by the interaction of a biweekly tidal cycle with temperature and salinity. Biweekly otolith check formation was found to be closely correlated with the phases of the moon, with or without the existence of an endogenous lunar rhythm. For the inshore lemon shark, lunar phases and tidal influences may play an important role in food availability, activity, stress, temperature and salinity, any of which could be a factor influencing circulus deposition.

The 3.4% APE index indicates that our ageing methods have a relatively high level of precision. This index compares favorably to indices of APE ranging from 0.3–6.3% found by Prince et al. (1985) for two readers examining internal bands on whole and sectioned vertebrae from Atlantic bluefin tuna, *Thunnus thynnus*. Al-

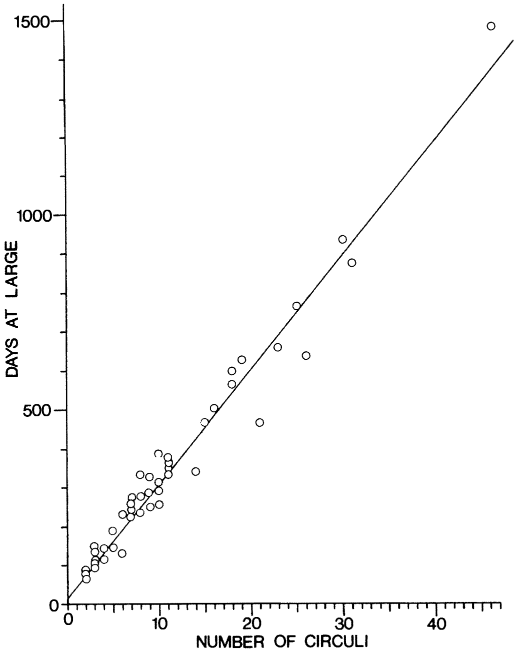

Fig. 4. The number of circuli deposited after TC marker deposition had a linear relationship to the number of days at liberty as represented by the equation: $Y = 29.21X + 23.02$, $r^2 = 0.97$, $n = 55$.

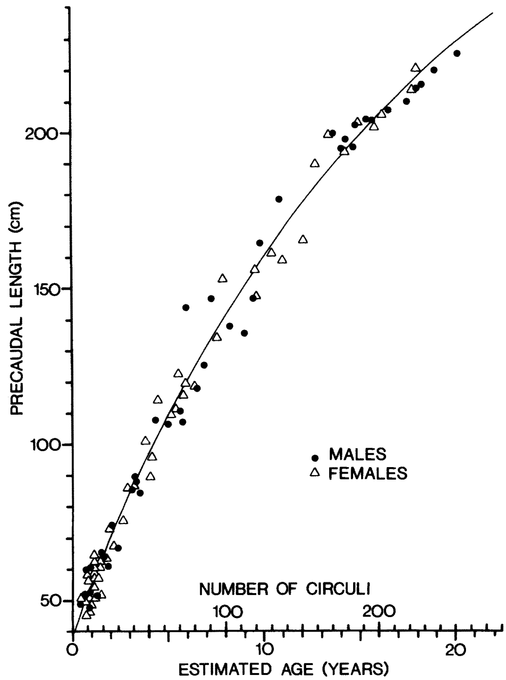

Fig. 5. Growth curve fitted to plot of PCL against circulus counts and corresponding estimated age according to the von Bertalanffy equation: $l_t = L_\infty (1 - e^{-K(t-t_0)})$, where l_t = PCL at time t, L_∞ = maximum theoretical length = 317.65 cm, K = growth constant = 0.057 and t_0 = theoretical age at 0 length = −2.302 yr. $r^2 = 0.99$, MSE = 37.9, n = 110. Parameters for each sex (not shown) were: for males L_∞ = 323.00, K = 0.055, t_0 = −2.314 and for females L_∞ = 310.10, K = 0.060, t_0 = −2.253.

though time-consuming, with each centrum requiring a minimum of 1–2 man-hours to process and read, this technique represents an accurate and precise approach to the age assessment of this species and warrants investigation in other species.

The predicted von Bertalanffy growth curve provides a close fit to the data points ($r^2 = 0.99$, mean square error = 37.9). The value of L_∞ exceeds the maximum size of 281 cm PCL reported by Gruber and Stout (1983) and the predicted size at birth differs slightly from the 46 cm PCL average obtained by Henningsen and Gruber (unpubl.) from full term and newborn pups. These values should be regarded as fitting parameters for the curve. Our predicted ages at maturity sharply contrast earlier estimates of 1.5–2.6 yr to maturity based on observations of wild and captive sharks (Springer, 1950; Clark and von Schmidt, 1965; Moss, 1972). This may be a result of small sample sizes, short experiment durations, and/or aberrant captive growth rates in previous studies. An accurate knowledge of age at maturity is extremely important, especially when dealing with species subject to increased exploitation. This study indicates that the lemon shark is late-maturing and lives at least 20 yr. Such knowledge of age and growth relationships is essential to understanding population dynamics, bioenergetics and the potential impact of fisheries.

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Literature Cited

Applegate, S. P. 1967. A survey of shark hardparts, p. 37–67. *In:* Sharks, skates, and rays. P. W. Gilbert, R. F. Mathewson and D. P. Rall (eds.). Johns Hopkins Press, Baltimore, Maryland.

Beamish, R. J., and D. A. Fournier. 1981. A method for comparing the precision of a set of age determinations. Can. J. Fish. Aquat. Sci. 38:982–983.

———, and G. A. McFarlane. 1983. The forgotten requirements for age validation in fisheries biology. Trans. Am. Fish. Soc. 112(6):735–743.

Beamish, R. J., and G. A. McFarlane. 1985. Annulus development on the second dorsal spine of the spiny dogfish (*Squalus acanthias*) and its validity for age determination. Can. J. Fish. Aq. Sci. 42: 1799–1805.

Cailliet, G. M., L. K. Martin. D. Kusher, P. Wolf and B. A. Welden. 1983. Techniques for enhancing vertebral bands in age estimation of California elasmobranchs, p. 157–165. *In:* Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes, and Sharks, NOAA Tech. Rep. NMFS 8. E. D. Prince and L. M. Pulos (eds.). 15–18 Feb. 1982. Southeast Fisheries Center, Miami Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Miami, Florida. U.S. Department of Commerce, Washington, D.C.

———, R. L. Radtke and B. A. Welden. 1986. Elasmobranch age determination and verification: a review, p. 345–360. *In:* Proceedings of the Second International Conference on Indo-Pacific Fishes. T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura (eds.). 29 July–3 Aug. 1985. Tokyo National Museum, Ueno Park, Tokyo, Japan. Ichthyological Society of Japan, Tokyo, Japan.

Campana, S. E. 1984. Lunar cycles of otolith growth in the starry flounder *Platichthys stellatus.* Mar. Biol. 80:239–246.

Casey, J. G., H. L. Pratt, Jr. and C. E. Stillwell. 1985. Age and growth of the sandbar shark (*Carcharhinus plumbeus*) from the western North Atlantic. Can. J. Fish. Aquat. Sci. 42:963–975.

Casselman, J. M. 1983. Age and growth assessment of fish from their calcified tissue—techniques and tools, p. 1–17. *In:* Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes, and Sharks. NOAA Tech. Rep. NMFS 8. E. D. Prince and L. M. Pulos (eds.). 15–18 Feb. 1982. Southeast Fisheries Center, Miami Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Miami, Florida. U.S. Department of Commerce, Washington, D.C.

Clark, E., and K. von Schmidt. 1965. Sharks of the central gulf coast of Florida. Bull. Mar. Sci. 15: 13–83.

Compagno, L. J. 1984. Sharks of the World. FAO Fisheries Synopsis No. 125, Vol. 4, Part 2, p. 519–520.

Gruber, S. H. 1982. Role of the lemon shark, *Negaprion brevirostris* (Poey), as a predator in the tropical marine environment: A multidisciplinary study. Fla. Sci. 45:46–75.

———, and R. G. Stout. 1983. Biological materials for the study of age and growth in a tropical marine elasmobranch, the lemon shark, *Negaprion brevirostris* (Poey), p. 193–205. *In*: Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes, and Sharks, NOAA Tech. Rep. NMFS 8. E. D. Prince and L. M. Pulos (eds.). 15–18 Feb. 1982. Southeast Fisheries Center, Miami Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Miami, Florida. U.S Department of Commerce, Washington, D.C.

Holden, M. J. 1974. Problems in the rational exploitation of elasmobranch populations and some suggested solutions, p. 117–137. *In:* Sea Fisheries Research. F. R. Harden-Jones (ed.). J. Wiley & Sons Inc., New York, New York.

———. 1977. Elasmobranchs, p. 187–214. *In:* Fish Population Dynamics. J. A. Gulland (ed.). J. Wiley & Sons Inc., New York, New York.

———, and M. R. Vince. 1973. Age validation studies on the centra of *Raja clavata* using tetracycline. J. Cons. Int. Explor. Mar. 35(1):13–17.

Jearld, A., Jr. 1983. Age determination, p. 301–324. *In:* Fisheries techniques. L. A. Nielsen and D. L. Johnson (eds.). American Fisheries Society, Bethesda, Maryland.

La Marca, M. J. 1966. A simple technique for demonstrating calcified annuli in the vertebrae of elasmobranchs. Copeia 1966(2):351–352.

MARQUARDT, D. W. 1963. An algorithm for least squares estimation of nonlinear parameters. J. Soc. Ind. Appl. Math. 2:431–441.

MOSS, S. A. 1972. The feeding mechanism of sharks of the family Carcharhinidae. J. Zool. (Lond.) 167: 423–436.

NATANSON, L. J., G. M. CAILLIET AND B. A. WELDEN. 1984. Age, growth and reproduction of the pacific angel shark (*Squatina californica*) from Santa Barbara, California. Amer. Zool. 24(3):130.

PANNELLA, G. 1971. Fish otoliths: daily growth layer and periodical patterns. Science 173:1124–1127.

———. 1974. Otolith growth patterns: an aid in age determination in temperate and tropical fishes, p. 28–39. *In:* Proceedings of an International Symposium on the Ageing of Fish. T. B. Bagenal (ed.). 19–20 July 1973. University of Reading, Reading, England. Unwin Bros. Ltd., The Gresham Press, Old Woking, Surrey, England.

PRINCE, E. D., D. W. LEE AND J. C. JAVECH. 1985. Internal zonations in sections of vertebrae from the Atlantic bluefin tuna, *Thunnus thynnus*, and their potential use in age determination. Can. J. Fish. Aquat. Sci. 42:938–946.

SMITH, S. E. 1984. Timing of vertebral band deposition in tetracycline-injected leopard sharks. Trans. Amer. Fish. Soc. 113(3):308–313.

SPRINGER, S. 1950. Natural history notes on the lemon shark, *Negaprion brevirostris.* Tex. J. Sci. 2:349–359.

URIST, M. R. 1961. Calcium and phosphorus in the blood and skeleton of the elasmobranchs. Endocrinology 69:778–801.

WILSON, C. A., E. B. BROTHERS, J. M. CASSELMAN, C. L. SMITH AND A. WILD. 1983. Glossary, p. 207–208. *In:* Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes, and Sharks. NOAA Tech. Rep. NMFS 8. E. D. Prince and L. M. Pulos (eds.). 15–18 Feb. 1982. Southeast Fisheries Center, Miami Laboratory, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Miami, Florida. U.S. Department of Commerce, Washington, D.C.

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