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Vertebral Growth Zone Deposition in Pacific Angel Sharks

LISA J. NATANSON AND GREGOR M. CAILLIET

Vertebrae and body size measurements were taken from 334 Pacific angel sharks collected from commercial gill netters off Santa Barbara, California, from Sept. 1979–Nov. 1983. Radiographs of vertebral centra from 247 specimens were studied to delineate calcified bands for age determination. The vertebrae of the smallest newborn sharks (260 mm TL) had 6 or 7 bands while those of the largest (1140 mm TL) had 42 bands. Bands were not deposited annually but were related to somatic growth. This hypothesis is supported by the number of bands in embryos and newborn Pacific angel sharks, growth of girth, and vertebral centrum dimensions, laboratory grow-out studies of tetracycline-injected sharks, and evidence from six tetracycline-injected tag returns.

THE Pacific angel shark, *Squatina californica* Ayres, is the only member of the family Squatinidae found off California (Bigelow and Schroeder, 1948; Herald, 1967; Compagno, 1984). This temperate species ranges from southeastern Alaska to Baja California and the Gulf of California and, perhaps, also from Peru to southern Chile (Walford, 1935; Roedel, 1953; Eschmeyer et al., 1983). The Pacific angel shark is reported to reach a maximum size of 1524 mm TL and 27 kg (Beebe and TeeVan, 1941; Miller and Lea, 1972; Castro 1983).

In 1976, a method was developed for filleting the Pacific angel shark; this led to the beginning of a commercial fishery in Santa Barbara. Commercial angel shark landings in Santa Barbara have increased from only 0.17 metric tons (MT) in 1977 to over 566.3 MT in 1985, and 561.3 MT in 1986 (J. Richards, 1987; pers. comm.) and have since declined to 426.4 MT in 1987 and 217.8 MT in 1988.

Most species of elasmobranchs studied have a late age at first reproduction, low fecundity, long gestation period, and slow growth (Holden, 1977). These factors combine to make elasmobranchs easily susceptible to overexploitation (Holden, 1977; Bedford, 1987). Therefore, to properly manage an elasmobranch fishery, it is imperative that aspects of the growth and age composition be known.

Vertebral centra were first considered for use in aging elasmobranchs by Ridewood (1921). Since then reliable techniques for elucidating vertebral band pairs have been developed and evaluated (Cailliet et al., 1983, 1986). Preliminary evidence on Pacific angel shark growth, however, suggested that they may not deposit annual band pairs. Young are born with 6–7 band pairs (Cailliet et al., 1983; Natanson, 1984) during an approx. 9 mo gestation period (Natanson and Cailliet, 1986). Though embryonic growth often does not represent postembryonic

growth in most elasmobranchs studied, one birth ring is deposited at parturition. In some cases, one or two light rings are formed prior to the birth ring, the former possibly representing events during gestation (Casey et al., 1985). However, in the case of the angel shark, six distinct, evenly-spaced band pairs were formed. This suggested that band pair formation in this species may be different than in those studied previously.

The objective of this study was to examine the age and growth of the Pacific angel shark off California. Four hypotheses concerning the periodicity of band pair formation were generated and tested: 1) band pairs are deposited annually throughout the life of the shark; 2) band pairs are deposited at a predictable but variable rate over the life of the shark; 3) band pairs are resorbed, or cease to form, in adult sharks, and/or at the head and tail region of the vertebral column; and 4) band deposition is related to somatic growth of the individual.

MATERIALS AND METHODS

Pacific angel shark specimens were collected off Santa Barbara, California, between Gaviota and Ventura from Sept. 1979–Nov. 1983. Most specimens were collected from commercial, 20 cm mesh, halibut gill or trammel nets operating at depths of 6–37 m or otter trawls at depths of 58–77 m.

Large specimens were dissected on board and small specimens were frozen for later dissection. Measurements (in mm) taken on most specimens were TL (measured from the tip of the head to the tip of the tail) and alternate length (AL, the distance from the origin of the first dorsal fin to the origin of the second dorsal fin). Three measurements of girth were also taken under the pectorals, above the first dorsal, and below the second dorsal.

As much of the vertebral column as possible was removed for use in ageing studies. To determine which vertebrae were consistently in the region of highest number ("plateau region"), counts were obtained for each vertebra along the entire column of 14 specimens (embryo—1180 cm TL). Band number was then plotted against vertebra number for each specimen and the plateau region determined. Only vertebrae numbers 12–14 were processed for ageing because they were the vertebrae with the highest band counts.

Vertebrae which were known to have been

taken from near the head and/or tail regions were not used in the age determination analysis. When vertebrae were taken from unknown areas of the vertebral column, X-radiography was used to detect morphologic characteristics to allow identification as head, trunk, or tail vertebrae (Natanson, 1984). Centrum width was plotted against TL and against band counts for vertebrae from positions 12–14, using the highest of the three centrum width and band count values from each specimen. A linear regression was then calculated and fitted to the points (Zar, 1974).

Both X-radiography and histology were used to delineate bands on vertebral centra (Cailliet et al., 1983). Whole vertebrae were X-rayed with a Hewlett-Packard Faxitron Series X-ray system (Model No. 43805N) with Kodak Industrex M film (ReadyPack M-2). Radiographs were examined under a dissecting microscope for counts and measurements. Two readers made separate and independent counts of the vertebrae on each radiograph. A band was defined as a single opaque or translucent concentric circulus, and this definition is similar to the definition of a ring by Cailliet et al. (1983). For readings that differed by only one band pair, the count of one reader was accepted for the first specimen and the count of the other reader was accepted for the next specimen with a difference of only one band pair. This alternation of accepting counts was continued until all the specimens with a difference of only one band pair were tabulated. If counts were off by two or more bands, the specimen was recounted by both readers, using a double-blind technique, until a consensus was reached.

For histological processing, 1–4 vertebrae from each column were removed, separated, and thawed. The vertebrae were then cleaned of excess tissue and preserved in 10% formalin for a minimum of 3 h. Centra were decalcified in 100% RDO, a commercial decalcifying agent (DuPage Kinetics, Illinois). The length of decalcification depended on the size of the centrum and ranged from 5 min to 8 h (Natanson, 1984). Centra were then trimmed, stored in 70% ethanol (ETOH), and embedded in polyethylene glycol (Natanson, 1984).

Sections were sliced on an A.O. rotary microtome between 10–15 microns thick. Large (>9.5 mm TL) Pacific angel shark centra were sliced longitudinally through the center, creating a half bow-tie shaped slice. The centra from smaller (<9.5 mm TL) specimens were

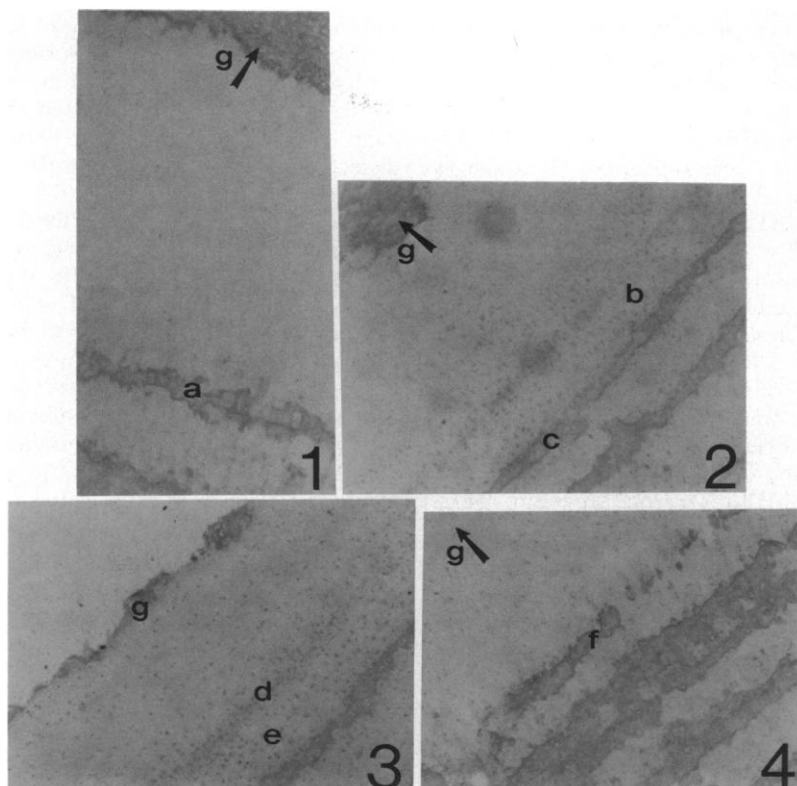


Fig. 1. Histological sections of the centrum edges of four specimens of *Squatina californica*. Each picture shows one of the four stages of band development used as a standard to judge edge development. Stages 1 and 2 represent translucent band development, and stages 3 and 4 represent opaque band development. 1(a) opaque band just formed, no growth beyond; 2(b) translucent band forming; (c) last opaque band; 3(d) opaque band forming; (e) translucent band formed; 4(f) opaque band forming; (g) edge. Photos were taken at 40 \times with an Olympus photomicroscopy system.

sliced transversely along the centrum face to create a larger section because a bow-tie section of the small size was more easily damaged in processing. Sections were stained in a 5% solution of Congo Red in 95% ETOH as recommended by Ridewood (1921) and then mounted with Permount.

To determine whether or not counts obtained from histology differed statistically from counts obtained from radiography for adjacent vertebrae, a simple linear regression through the origin was calculated (Zar, 1974). Slopes of lines were compared by t-test and if the slopes were not significantly different at the 0.05 probability level, the methods were considered to produce equivalent counts.

Centrum edge development was divided into four categories on the basis of the amount of opaque or translucent band information (Fig.

1). Stage 1 represented the beginning of formation of a translucent band. The last opaque band had been formed and a few individual cells were visible after it. Stage 2 represented further formation of the translucent band. At this stage more cells have filled the band area and band width was defined. Stage 3 represented the beginning of opaque band formation. Clumps of cells now gathered at the cell proliferation zone. Stage 4 represented the further development of the opaque band. At this stage more cells were visible and the band was becoming more dense.

Samples of Pacific angel shark vertebrae, taken monthly from June–Oct. 1982, and taken weekly from Nov.–Dec. 1983, were processed histologically to discern the changes in band disposition patterns over time for centrum edge analysis. Centra from along the whole vertebral

column of one angel shark were sectioned histologically to see if the edges of centra from all parts of the body had the same edge development.

Whenever possible, small (<800 mm TL) Pacific angel sharks were kept alive. These sharks were transported to the Moss Landing Marine Laboratories (MLML) and maintained together in a 2.5 × 1.2 × 0.5 m wooden tank behind a plywood enclosure and subject to natural photoperiod. The tank was equipped with constantly circulating ambient Monterey Bay seawater pumped from a beach well, aerators, and a 5 cm deep layer of fine sand.

The sharks were primarily fed thawed anchovies and occasionally thawed squid and mackerel, using a method modified from R. Johnson (Cabrillo Marine Museum, pers. comm.). Sharks were fed *ad libitum* once each weekday for 5 min. Uneaten food was removed thereafter. In addition, live juvenile rockfish, smelt, and sticklebacks, when available, were placed in the tank so that the sharks could feed on their own schedule.

The live sharks were initially measured, weighed on a bench beam scale, sexed, and injected with a 25 mg/kg of body weight (BW) dose of tetracycline (OTC) a few days after capture. After the initial measurements, the fish were measured in the tank to reduce trauma. Information on TL and food intake was logged while the sharks were alive.

Upon death, sharks were measured and dissected in the manner previously described. Because tetracycline loses intensity with exposure to light (Weber and Ridgway, 1962), the dissections were done in a darkened area. To delineate the tetracycline mark as well as the bands, vertebrae were processed using a resin embedding method (Smith, 1984). Resin embedded samples were sectioned longitudinally along the widest part of the oval to approx. 0.2 mm. Sections were then attached to a microscope slide with clear enamel nail polish and viewed under a dissection microscope in the dark with a short-wave (365 nm) portable ultraviolet light to illuminate the tetracycline mark. To determine if tetracycline was incorporated into all centra of an individual, every fourth centrum along the column of the laboratory-grown sharks was sectioned and examined for fluorescence.

In Oct. 1981 and March–April 1983, 17 and 88 live Pacific angel sharks from commercial gill nets were sexed, measured, tagged, injected

with a dose of 25 mg/kg BW of tetracycline, and released alive off the coast north of Santa Barbara and the Channel Islands, respectively. To date, six tagged and injected angel sharks have been recaptured. Their vertebrae were dissected and analyzed as were the laboratory-grown specimens.

Mean growth rates (mm/month) were calculated for the two laboratory-grown newborn sharks. Mean monthly growth was added to mean size at birth and plotted against months, adding the mean growth value to the lengths at each successive month, to show growth for the first year of life. The growth rate for the second year of life was obtained from information gathered on a shark kept at the Cabrillo Marine Museum for over 24 mo (R. Johnson, pers. comm.). Also, growth rates were calculated for five of the six recaptured sharks.

Growth rates were calculated for laboratory-grown and field-tagged fish. A mean value for the growth in mm per band pair deposited in the laboratory-grown sharks was calculated. This was then superimposed on the TL band number graph, starting at the mean TL and number of bands at birth up to the maximum number of bands laid down by our live sharks. Similar values were calculated for those tag recaptured sharks which had the necessary information.

Girth measurements were made along three regions of the body and plotted against TL to give an estimate of relative girth growth along the body. Regressions were calculated and an analysis of covariance was performed, followed by multiple comparisons among slopes (Zar, 1974). The differences between the lines were then related to the changes in band number at these regions of the body.

RESULTS

Pacific angel shark vertebrae were generally oval but varied in morphology in differing parts of the body (Natanson, 1984). Trunk vertebrae could be distinguished from head and tail vertebrae by the position of the basapophyses (terms following Walker, 1975); however, not all trunk vertebrae were from the plateau region. Vertebrae 12–14 were consistently in the plateau region. Trunk vertebrae, numbers 8–48, were indistinguishable by morphology. Calculations of percent deviation of unknown vertebrae from those consistently in the plateau region (12–14) showed that the range of deviation in band

counts of the unknown trunk vertebrae would be 0.5–2.43 bands in a shark which had 31 bands in its plateau vertebrae (only a 1.6–7.8% difference). This deviation was acceptable, and it was felt that the trunk vertebrae that could not be differentiated by morphology were within a reasonable range for use in ageing.

Band counts differed along the vertebral column, and were especially variable in larger fish (Fig. 2). Counts were low in centra taken from immediately behind the head, increased to a plateau, and decreased towards the tail (Fig. 2). In embryonic individuals, counts only varied by one but with larger sharks, the differences were higher and more variable. After examining band counts along the column of 14 individuals (embryo to 1180 mm TL), it was determined that the 12th–14th vertebrae were consistently within the plateau of highest counts. There was a significant linear relationship between TL and centrum width ($r^2 = 0.98$) and between band number and centrum width ($r^2 = 0.92$) for vertebrae 12–14.

Band counts agreed well on centra from vertebrae on all sized specimens processed with both radiographic and histological techniques. Two readers counted centra processed with both methods on 111 samples of specimens ranging from 210–1170 mm TL. For radiography, 102 (57%) of the initial counts were the same, 52 (29%) disagreed by one, and 23 (14%) disagreed by two or more bands. For histology, 57 (50%) of the initial counts were the same, 42 (38%) differed by one, and 14 (12%) differed by two or more band counts. There was no significant difference in counts between the two techniques ($t_{0.05} = 0.379$); therefore, counts made using both methods were used interchangeably in data analysis.

Girth increased with size more rapidly at the region just below the pectoral fins than at the two tail regions (Fig. 3). Analysis of covariance revealed that there was a difference among the three lines ($F_{0.05} = 1180.31$). The results of the multiple comparisons among slopes indicated that all three slopes were different (lines 1–2, $q_{0.05} = 9598$; lines 2–3, $q_{0.05} = 13,880$; lines 2–3, $q_{0.05} = 4282$).

For both males and females, the number of bands increased with increasing length until 900 mm TL (Fig. 4). The graph for males is basically linear but seems to level off after 1000 mm TL, although the sample size in this region is small. The graph for females is linear until 700–800

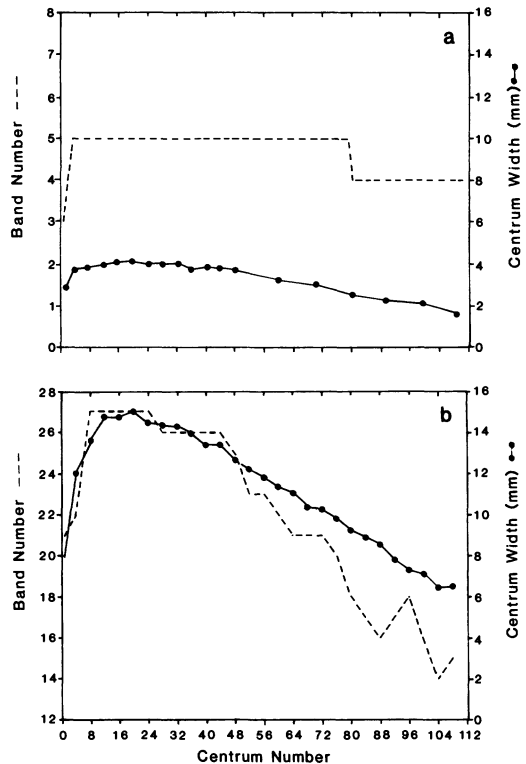


Fig. 2. Graph depicting the relationship of band number and centrum width with centrum number of every fourth vertebra along the vertebral column of (a) a 225 mm TL specimen, and (b) an 850 mm TL specimen.

mm TL, when a more rapid increase in size is evident. After 1000 mm TL there is considerable variability among the points rather than a leveling off.

Edge formation was not predictable by time of year. The majority (102) of the 111 specimens collected from June–Oct. 1982 and in Nov. 1983, which were examined histologically for band development at the centrum edge, were in developmental stages 1–2 (Fig. 1). For all sizes of fish, stages 3–4 (opaque band formation) were observed more often in winter samples than summer samples but fish in these stages were still low in number and the occurrence of this stage was not predictable by time (Table 1). No lunar periodicity was noted from the edges of centra from angel sharks over 900 mm TL collected weekly. Only one fish out of 15 taken over the 4 wk period was in stage 4; all others were in stage 1. Examination of every fourth

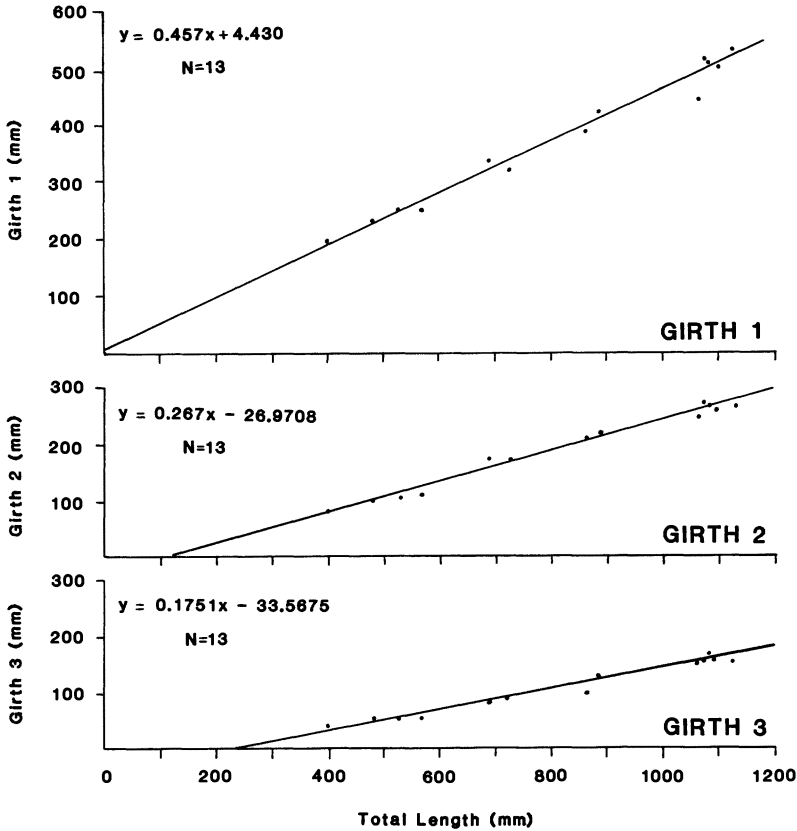


Fig. 3. The relationship between girth (mm) and TL for 13 angel sharks with associated linear regressions: Girth #1—under the pectorals; Girth #2—above the first dorsal; Girth #3—below the second dorsal.

vertebra from the column of a 1180 mm TL female collected in Jan. 1983 showed that although there were differing numbers of bands in each centra, all growing edges were in stage 1.

Tetracycline was incorporated in all centra of the vertebral columns of the three laboratory-grown, injected individuals (Table 2). All parts of the columns exhibited fluorescence under ultraviolet light and, therefore, had deposited tetracycline. Also, all exhibited band growth following the mark.

Laboratory specimen number 951 measured 592 mm TL on 30 Oct. 1982 when caught and 615 mm TL on 8 Dec. 1982 when it died. It had grown a total of 23 mm in 5 wk and had a growth rate of 18.4 mm/mo. This shark decreased 0.15 kg in weight (from 1.42 kg–1.27 kg), and it never fed in captivity. Sections of centrum number 12 showed a discrete fluorescent mark in the 19th opaque band (Fig. 5). The resin embedded centrum section showed

growth of a translucent and the beginning of an opaque band in the region outside the tetracycline mark.

The two longest lived sharks (specimens 285 and 333) were postpartum pups originally removed from a pregnant female on 29 March 1983. Specimen number 285 was 254 mm TL when first measured on 14 April 1983 and 348 mm TL when it died between 2–5 Oct. 1983. It had grown 94 mm in 6 mo and had a growth rate of 15.67 mm/mo (Table 2). However, there was a decrease in growth rate following the tetracycline injection on 5 July 1983 (Fig. 6). This was followed by a rapid rate of growth in Sept. and Oct. Upon dying, it had 10 total opaque bands on centrum number 10 (centra 12–14 were damaged severely when the shark died and therefore were unusable), three of which were deposited in the approx. 3 mo since the tetracycline mark. Specimen 333 was 250 mm TL and weighed 168 g on 14 April 1983 when first measured and 412 mm TL and 462 g when it

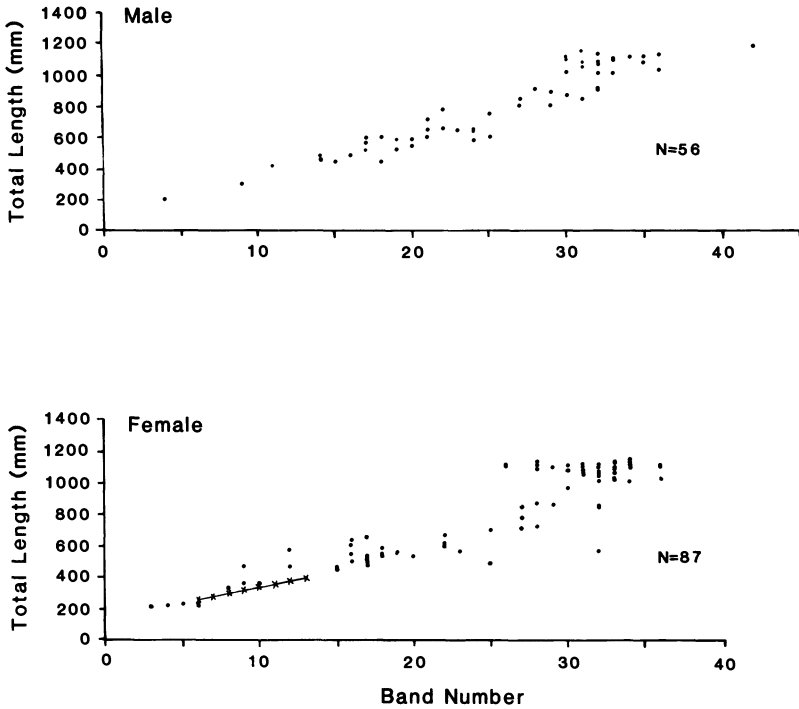


Fig. 4. The relationship between TL (mm) and number of bands for 56 male and 87 female angel sharks. The solid line connecting the x's represents the size of known-age captive angel sharks from birth, at which time there were five bands in the vertebral centra (Natanson, 1984).

was sacrificed on 21 May 1984. It had grown 162 mm (a rate of 12.5 mm/mo) and gained 294 g (Table 2). The growth of this specimen appeared to be unaffected by the tetracycline injection (Fig. 6). This shark was injected with tetracycline on 24 May 1983. Upon dissection it had a total of 13 opaque bands on centrum number 12, seven of which were deposited in the 12 mo after the tetracycline injection. These sharks (specimens 285 and 333) started to feed 41 d after capture. Until this time the sharks were using internal yolk reserves which were visible through the ventral skin. The yolk decreased in size from time of capture to initiation of feeding. After feeding was initiated, the sharks generally fed once every 2 wk for 1.5 mo and then fed once or twice a week.

The number of vertebral centrum bands present at birth was obtained from four other pups removed from the same pregnant female angel shark on 29 March. One of the young died on 29 March and had five bands on centrum number 12. Three more died within a month after capture and had 5+ to 6 bands on centrum number 12. Because all were approx-

imately the same size and in the same developmental stage at capture, it was assumed that they all had five bands at capture. Each of these young sharks also had internal yolk sacs. The shark that died on 29 March had the largest, and each successively dying shark had decreased yolk in the sac indicating that it was being used for nutrition.

Growth and band deposition rates for our laboratory-held fish specimens 285 and 333 were compared to the same information from a newborn Pacific angel shark maintained for 11 mo at California State University, Long Beach (CSULB) (J. McKibben, pers. comm.) (Table 2). This shark was measured initially at 240 mm TL on 8 April 1982. At this time, according to our band number and size data (Fig. 4), the shark would have had approximately six band pairs. The shark died 11 mo later (13 May 1983) at 290 mm TL, having grown 50 mm and having eight bands, apparently adding only two band pairs.

These findings indicated that there was a closer relationship of band deposition to growth in TL than to time. The MLML grown angel sharks

TABLE 1. FREQUENCY OF HISTOLOGICAL CENTRUM EDGE STAGES BY MONTH OF 120 ANGEL SHARKS COLLECTED OFF SANTA BARBARA, CALIFORNIA.

	June 1982	July 1982	Aug. 1982	Sept. 1982	Oct. 1982	Nov. 1983
Stages 1-2	0.94	0.75	0.79	0	0.50	0
Stages 3-4	0.06	0.25	0.21	0	0.50	0
n	16	8	19	0	14	0
Size range: 210-860 mm TL						
Stages 1-2	0.92	0.89	0	0	1.0	0.62
Stages 3-4	0.08	0.11	0	0	0	0.38
n	36	18	0	0	1	8
Size range: 915-1170 mm TL						

grew 12.46-15.67 mm/mo, while that grown at CSULB grew 4.55 mm/mo. Also, the MLML sharks deposited between 0.54-0.83 band pairs/mo while the CSULB shark deposited only 0.18-0.27 pairs/mo.

Specimens 285 and 333 added one band pair for every 20 mm TL. Assuming that this is the rate of band deposition for the first 13 bands (the maximum of specimen 333), the resulting growth line (Fig. 4) fits closely with the field-collected data on size and band number. Using the mean growth per month for specimens 285 and 333 (13.54 mm), and adding the average length at birth (260 mm TL) a 1 yr old shark would be approx. 422 mm TL.

Field growth rates were variable (Table 3). When measured as the growth per month in millimeters, the range was from 0.09-3.21, considerably lower than that exhibited by the laboratory-grown animals (Table 2). Calcification processes were occurring because tetracycline was observed in all centra along the entire vertebral column of all six fish. The number of bands which took up tetracycline varied from 1-4, always in the outer bands (Fig. 7). Assuming that the outermost band (which in all cases showed the most fluorescence) deposited the tetracycline at time of injection, the number of additional bands should indicate subsequent

calcification activity. Three recaptured fish had no additional band pairs at the periphery of their centra while the others ranged from 1-3 band pairs (Table 3).

When there was a variable number of additional bands noted, the highest number occurred in the largest trunk vertebrae (numbers 10-20) while the lower numbers were in the tail region. The four largest individuals (980-1151 mm TL at recapture) exhibited little growth, ranging from 0.09-3.21 mm TL/mo, and had low fluorescence, indicating that little calcification, if any, was occurring in their centra. The smaller individuals also grew slowly, especially when compared with the limited laboratory growth data (Table 2), but tended to deposit more new calcified bands. Of the long-term recaptures (over 3 yr), the large one (specimen 336) grew little and added no calcified bands, while the smaller one (specimen 335) grew somewhat more and added 2-3 band pairs, depending upon position along the vertebral column (Fig. 7).

DISCUSSION

The data indicate that angel sharks do not deposit vertebral bands annually, or in a temporally predictable manner, thus making it im-

TABLE 2. GROWTH CHARACTERISTICS OF LABORATORY-GROWN SPECIMENS REARED FROM BIRTH AT MLML AND CSULB.

Specimen No.	Size at birth	No. of bands at birth	Change in size	Additional bands	Age	Growth in mm/mo	No. of band pairs/mo	No. of band pairs/yr	mm/band pair
MLML 285	254 mm	5	+94 mm	5	6 mo	15.67	0.83	9.96	19
MLML 333	250 mm	5	+162 mm	7	13 mo	12.46	0.54	6.48	20
CSULB	240 mm	5-6	+50 mm	2-3	11 mo	4.55	0.18-0.27	2.16-3.24	25-17

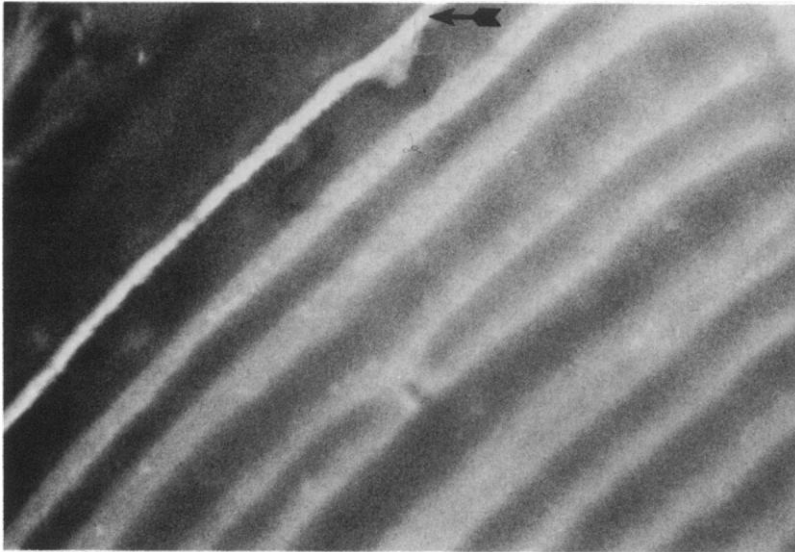


Fig. 5. Photograph of tetracycline mark (arrow) and subsequent band growth to the left of the mark of specimen 951. Photograph was taken using an Olympus photomicroscopy system with a dissecting microscope. Lighting was provided by a U.V. light source.

possible to age individual fish using vertebral bands. This is in contrast to the majority of other studies in which elasmobranch age determination and validation has been attempted (Cailliet et al. 1983, 1986). In these studies, most have shown that one pair of bands is deposited

annually throughout the life of the fish, as in the Japanese skate (*Raja fusca*) (Ishiyama, 1951), the thornback ray (*R. clavata*) (Holden and Vince, 1973), and the leopard shark (*Triakis semifasciata*) (Smith, 1984).

The number of bands varies considerably

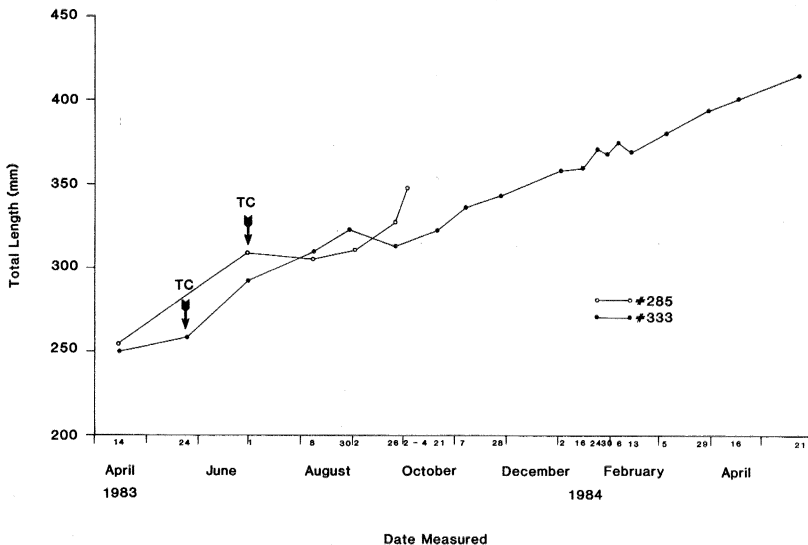


Fig. 6. Growth of laboratory-grown specimens 285 and 333. Time of tetracycline injection is indicated by arrows.

TABLE 3. GROWTH CHARACTERISTICS OF FIELD TETRACYCLINE-INJECTED, TAGGED-RECAPTURED SPECIMENS.

MLML No.	Date		Time at large	TL (mm)		Growth (TL)		Total number bands	Bands with OTC	Additional band pairs*	Band pairs/mo	Band pairs/yr	mm./band pair
	Tagged	Recaptured		Tagged	Recaptured	mm	mm./mo						
334	7-8 May 85	15 Jan. 86	8.3 mo	?	772 mm	?	?	25-27	3-4	0-2	0-0.24	0-2.88	?
335	14 July 82	21 Jan. 86	42.2 mo	652 mm	710 mm	58 mm	1.37	22	2-3	2-3	0.04-0.07	0.48-0.84	19-29
336	13 July 82	22 March 86	44.5 mo	970 mm	980 mm	10 mm	0.23	32	1	0	0	0	0
338	6 May 85	22 March 86	10.5 mo	1120 mm	1151 mm	31 mm	2.95	32	1	0	0	0	0
337	6 May 85	25 March 86	10.6 mo	1102 mm	1136 mm	34 mm	3.21	34	1	0	0	0	0
339	7 May 85	20 April 86	11.4 mo	1129 mm	1130 mm	1 mm	0.09	30	1	0-1	0-0.09	0-1.08	0-1.08

* Additional band pairs refers to centrum band pairs formed distal to the tetracycline mark.

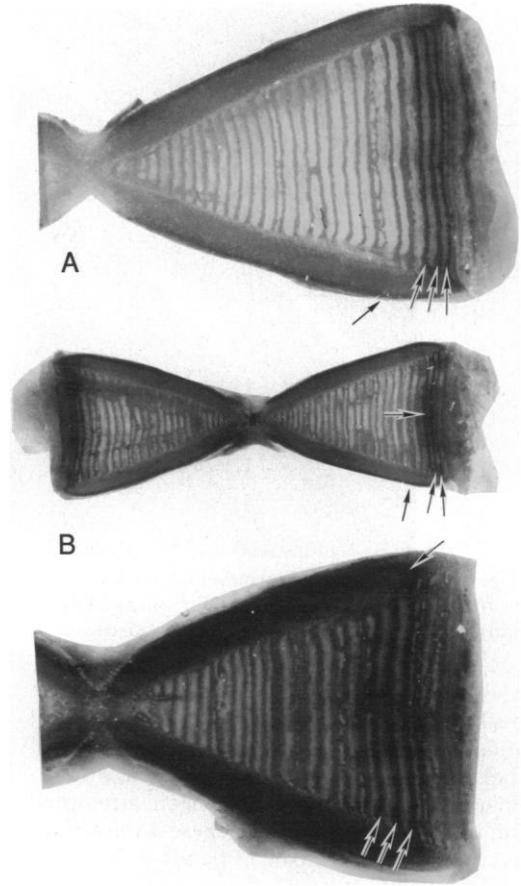


Fig. 7. Photographs of sectioned vertebral centra from recaptured angel sharks with tetracycline incorporation: (a) specimen 334, centrum number 12, showing 3 bands with tetracycline, and 2 subsequent bands; (b) specimen 334, centrum number 50 (nearer the tail), showing 2 bands with tetracycline, and no additional bands; and (c) specimen 335, centrum number 10, showing 3 bands with tetracycline, and 2 subsequent bands. Tetracycline bands are labeled with arrows.

along the vertebral column of the angel shark (Ridewood, 1921). In young sharks, OTC was deposited all along the column, but in larger individuals there was often less OTC and there were fewer subsequent bands deposited, the numbers of which varied depending on the position the vertebra had in the column. This most likely reflects the change in girth with size of fish and indicates that vertebrae grow in diameter only when the body being supported by the column increases (Fig. 3).

Other results also indicate that angel sharks do not deposit band pairs annually. The centrum edge histology results on adult Pacific angel sharks show that it is not possible to predict at what time of year the opaque and/or translucent bands are forming. This is in contrast to Japanese black skate, *R. fusca*, in which an annual band pair formation pattern was demonstrated (Ishiyama, 1951). Data from the three tetracycline-injected, laboratory-grown young Pacific angel sharks indicated that band deposition in these sharks was not annual. This is further supported by the information from the CSULB shark that added two band pairs in 11 mo. Also, 5–7 band pairs occur in newborn angel sharks (Cailliet et al., 1983), and monthly analysis of mean sizes of embryos suggests a gestation period of approx. 10 mo (Natanson and Cailliet, 1986). Therefore, embryonic band pairs are certainly formed more frequently than once per year.

The second hypothesis that band pair deposition rates are predictable but vary over life cannot be totally rejected or supported. Data from laboratory-grown and tag-recaptured sharks indicates that growth is faster and more bands are deposited in younger fish than in older, larger fish (Tables 2–3). Band deposition appears to be predictable based on growth (Table 2) but not time. Even though there was a significant relationship between band number and TL, the high variability in band counts of similar-sized adults obscures this relationship.

Our data both support and contradict the third hypothesis that band pairs might be resorbed or cease to form in adult sharks and/or at the head and tail region of the vertebral column. Our histology results on centrum edges over seasons and the fact that tetracycline is deposited at the edge of all centra in the column of laboratory-grown and field-recaptured angel sharks do not support this hypothesis. Band formation (i.e., calcification) was occurring in all centra examined. If active resorption was taking place, chondroclasts would be required to resorb the calcium from the broken-down bands, (Urist, 1961) and this was not observed. If bands were not forming, new band cells would not be present; however, new cells were found in all sections examined. All centra examined could be placed in one of the four formation categories of bands; this also indicates that bands were forming and resorption was not occurring. However, in the larger field-recaptured sharks, the amount of tetracycline deposited was low

and in some cases there were bands deposited in anterior vertebrae but not in those nearest the tail. This indicates decreased calcification activity at the posterior regions but does not necessarily suggest resorption. The lack of band pairs deposited past the OTC mark in the posterior vertebrae supports the hypothesis that band pair deposition is related to growth. Due to the slower rate of growth in the girth in the posterior of the shark, additional support in the vertebrae from this area may not yet have been necessary.

Examination of the relationship between TL and band number shows that a change in either growth or band deposition occurs between 800–1000 mm TL (approx. 28 bands) (Fig. 4). There was more variability in band counts of adults than young and more variability in centrum size and band counts along the vertebral column in adults. This area of the curve corresponds to the length when maturity is reached by both males and females (Natanson and Cailliet, 1986), but the change in growth is most noticeable in the female. One explanation could be resorption of calcium (and subsequent band loss) to route calcium to the growing embryos. Another possibility is that the high variability in number of bands may be caused by a decrease in deposition during reproduction.

The fourth hypothesis, that band deposition in the Pacific angel shark is more closely related to somatic growth than time or age, is supported by most of our results, especially the grow-out studies of young live fish, data from the tag and recapture program off Santa Barbara, band number at birth as related to gestation period, and results of girth measurement analysis. Data from the young Pacific angel shark grown at CSULB, combined with the grow-out data from this study, indicate that band deposition is related to body growth rather than to time. Because this shark was not injected with tetracycline, this could not have contributed to the slow deposition of the bands. By comparing the data on band deposition of our live sharks to the CSULB data and recapture data, it appears that band number may be dependent on somatic growth and not time (Table 2). One of our sharks (specimen 285) deposited more bands in a shorter period of time and had a faster growth rate than the CSULB shark. There was a dip in growth after the OTC injection in this shark, but this could be due to inaccuracies associated with measurement or individual variation (Fig. 6). Our other shark (specimen 333) deposited

more bands, lived longer, and grew more than either specimen 285 or the CSULB shark, but still did not deposit the bands in a temporally predictable manner. The size and band numbers of all three of these sharks fit perfectly on our field band number and size graph (Fig. 4). Both of the MLML sharks deposited one band pair for each 20 mm of TL, but bands were not deposited on any predictable time frame. This indicates that band pair deposition is related to somatic growth rather than to time.

The relationship of rate of increase of girth to centrum size supports the hypothesis that band deposition is related to somatic growth. The area with the highest increase in girth corresponded with the plateau area, and the areas of slower increase corresponded to the areas well outside the plateau area. This shows a relationship between body mass growth and centrum growth. Because band number increases linearly with centrum width, band number is also related to body growth. This, perhaps, suggests that we should measure growth of angel sharks using disk width, rather than TL, because growth of older fish is most likely in that dimension rather than length.

It has been well documented that rate of growth in captive sharks is higher than growth in the field. Gruber and Stout (1983) found that lemon sharks grew almost nine times faster in captivity and Wass (1973) found that grey reef sharks grew 10 times faster in captivity than in the field. In the present study, the captive sharks appeared to grow faster than the tagged sharks. If band pair deposition was strictly related to time, a difference in growth rate would not effect deposition. It is apparent from the captive shark data presented that deposition is not related to time. The field data, though variable, support this finding.

The relationship between band pair deposition and only somatic growth has not previously been documented in elasmobranchs (Cailliet et al., 1986). In other elasmobranch ageing studies that have been validated, band pair deposition has been found to be related to time. Tetra-cycline has been used to validate the annual periodicity of band pairs in the lemon shark (*Negaprion brevirostris*) (Gruber and Stout, 1983), the leopard shark (*Triakis semifasciata*) (Smith, 1984), and the thornback ray (*R. clavata*) (Holden and Vince, 1973); and histology was used to validate the annual periodicity in the Japanese skate (*R. fuscus*) (Ishiyama, 1951). It has been hypothesized that the bands may be de-

posited on this annual cycle due to seasonal temperature, food, migrations, and/or ion changes in the environment (Ridewood, 1921; Jones and Geen, 1977). In the Pacific angel shark's situation, where band pairs appear to be deposited relative to somatic growth, it is possible that the more heavily calcified bands may be deposited to strengthen the vertebral column (Ridewood, 1921).

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