Age-validation of a leopard shark (*Triakis semifasciata*) recaptured after 20 years

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On 10 July 1999, vertebrae bearing an oxytetracycline (OTC) time mark were retrieved from a tagged leopard shark *(Triakis semifasciata)* recaptured in San Francisco Bay, CA, after being at liberty for almost 20 years. An additional long-term leopard shark tag return was received in June 2001, for which growth information (but not vertebrae) was obtained. The first recapture is significant in that it represents the longest at-liberty period for an age-validated (OTC-injected) shark, extends and completes age validation for this species, spanning all age classes up to its estimated average maximum age, and provides an example of the persistence of the OTC time mark in an elasmobranch at liberty for almost 20 years. The recaptured leopard shark made in 2001 also provides valuable information on long-term growth from time of release to time of recapture. Findings are documented here so that other researchers are aware that validation is complete for this species, to present pertinent evidence of considerable interannual variability in growth in this species, and to report observations on processing difficulties relating to the ephemeral nature of the 20-yr-old OTC mark.

Earlier results from the tagging study that led to these recaptures were published by Smith (1984), Smith and Abramson (1990), and Kusher et al. (1992). These authors concluded that one pair of vertebral growth bands (one opaque, one translucent) is produced each year in this species for the age classes they examined (up to 17 years). Opaque bands are deposited primarily in late spring and summer (mainly in August in the San Francisco Bay area); translucent bands, presumably representing minimum growth periods, are deposited primarily in late fall and winter (Kusher et al., 1992). Average annual growth of recaptured leopard sharks examined in these studies ranged from 0.0 cm per year to 4.0 cm per yr (mean of 2.14 cm per yr); centra grew proportionately to shark length over all size classes sampled.

The leopard shark occurs from Baja California, Mexico, to Oregon; maximum confirmed size is 180 cm TL (Kato et al., 1967), but fish over 146 cm TL are uncommon off central California (Herald et al., 1960; Kusher et al., 1992). Age and size at which females first reach maturity have been estimated at approximately 13 yr and 110 cm TL, respectively; the gestation period is estimated at 10–12 months; and parturition takes place in spring (April–May peak) in San Francisco Bay (Smith and Abramson 1990; Kusher et al. 1992).

Methods

The recaptured sharks were from a group of 948 leopard sharks tagged and released off Hunterís Point (37°44′N lat., 122°21′W long.) in South San Francisco Bay between 26 July and 13 September 1979. All were injected with oxytetracycline hydrochloride (OTC), tagged on the dorsal fin with yellow plastic livestock rototags, and released as described by Smith (1984).

The shark returned in 1999 was caught on 10 July off Oakland Airport in San Francisco Bay by a recreational angler fishing ~8 km east of the original release point. The fish was measured and the vertebrae removed and later frozen. In the laboratory, the vertebrae were sectioned and mounted as described by Smith (1984). Various vertebral section widths (from 0.4 mm to 1.3 mm wide) were tested to determine the best thickness for interpreting narrow band patterns near the centrum edge. The transverse sections were viewed illuminated alternately by ultraviolet UV (365 nm) and transmitted light. Micrographs were taken with a Leica DMLB microscope with a Diagnostic Instruments SPOT CCD (charged coupled device) digital camera coupled through a 0.6× phototube, multiplied by the ocular magnifications of 1, 5, and 10× to obtain a total magnification of 0.6, 3, and 6×. The three magnifications were evaluated for the best possible counting path. The images were then analyzed with Media Cybernetics Image Pro Plus image analysis software (Media Cybernetics, 1998), with which increments were enhanced with an edge-sharpening filter. After the application of the filter the images were evaluated and counts from the OTC mark to the margin were made. An annulus was defined as the distal edge of each translucent band in the corpus calcareum, at the distal edge of each band pair (e.g. Kusher et al. 1992; Branstetter and Stiles 1987 and

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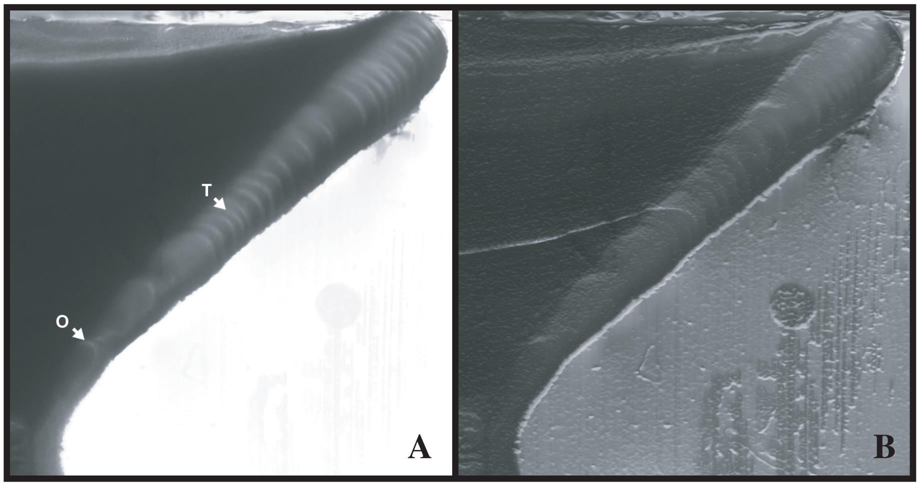

Figure 1

Photomicrographs of a transverse section of a vertebral centrum from a leopard shark (*Triakis semifasciata*) recaptured 10 July 1999 after ~20 years at liberty. (**A**) Transverse section viewed with transmitted white light showing broad dark opaque and narrow light translucent growth zones and ages up to year of tagging; and (**B**) the same section viewed with reflected ultraviolet light showing the OTC mark. 0 = first winter annulus, T = tagging and OTC injection of late summer 1979.

others). The first translucent band was interpreted as the first winter growth after parturition (year 0); the subsequent annulus interpreted as year 1. Band pairs distal to the tetracycline mark were compared with the number of years that the fish had been at liberty. For publication, digital photomicrographs were further enhanced to improve contrast and definition with Jasc Paintshop Pro software (Jasc Software, Inc., 1999).

Growth information for the other tagged fish (male, tag no. 337, recaptured in South San Francisco Bay on 9 June 2001) was obtained by comparing tagging and recapture size. Age and growth information for both recaptured sharks was compared with Kusher et al.'s (1992) von Bertanlanffy growth curves (their Figs. 4, 9, and 10) based on aged vertebrae from 1) fishery and market samples, and 2) earlier recaptures from the same 1979 tagging experiment.

Results

The shark recaptured in July 1999 was a mature female measuring 124 cm TL at recapture. At liberty growth and exact time at liberty could not be calculated because the tag number had worn away, preventing referral back to original tagging length and day. Because the one-time tagging experiment took place 26 July–13 September 1979, the fish had been at liberty from 19.8 to 19.9 years. The tetracycline mark was visible as a thin yellow line in all vertebral sections examined (Fig. 1). In less than a minute, the fluorescence began to decay rapidly, much more rapidly than tetracycline marks observed by the senior author in previous recaptured sharks (sharks at liberty 7 years or less). This fast decay made viewing of actual samples brief; most time was dedicated to setting up and taking digital photomicrographs of the sections.

Twenty annuli up to winter 1998 (including the 1979–80 winter annulus deposited after tagging) were visible distal to the time mark (Fig. 2), including a partially formed opaque band interpreted as the 1998 summer band (Fig. 3). We could not determine with any certainty whether the narrow opaque edge represented the beginning of the summer 1999 band (Fig. 3). On all sections examined, the last band pair at the centrum edge (i.e. the most recently deposited) was very narrow and faint, or incomplete and difficult to differentiate, especially on vertebral sections that were sliced too thick or too thin (best thickness was 0.6 mm). Proximal to the OTC mark were five annuli, indicating that the fish was 4+ yr old at tagging and was probably born in spring of 1974. The fish was aged as 24+ or in its 25th year. Width of the opaque bands indicative of annual summer growth was highly variable, although it generally tended to decrease with increasing age. Greatest relative growth in centrum diameter occurred in the first few years of life, and also in 1985, 1986, and 1987, judging from the width of the corresponding opaque zones (ages 11, 12, and 13). The recapture size (124 cm TL) was 11 cm smaller than that predicted for an age-25 female with the von Bertalanffy growth function (VBGF) generated by Kusher et al. (1992, their Fig. 4) for 162 untagged leopard sharks. But it was close to that predicted by their FISHPARM VBGF generated from band counts and lengths of tag recaptures from this same 1979 experiment (Kusher et al. 1992, their Fig. 10).

The other recaptured leopard shark (a male, no. 337) was caught 9 June 2001 in South San Francisco Bay off

San Mateo, CA, making a total of 120 recaptured leopard sharks from the original 1979 tagging. The tag number was readable and traceable back to the tagging date (23 August 1979) and length (87 cm TL), which was compared with the recapture length (119.4 cm TL) and date. This individual exhibited an average annual growth of only 1.47 cm/yr, but its recapture length for its presumed age (30) was also close to that predicted by the Kusher et al. (1992) asymptotic FISHPARM VBGF for tagged and recaptured leopard sharks.

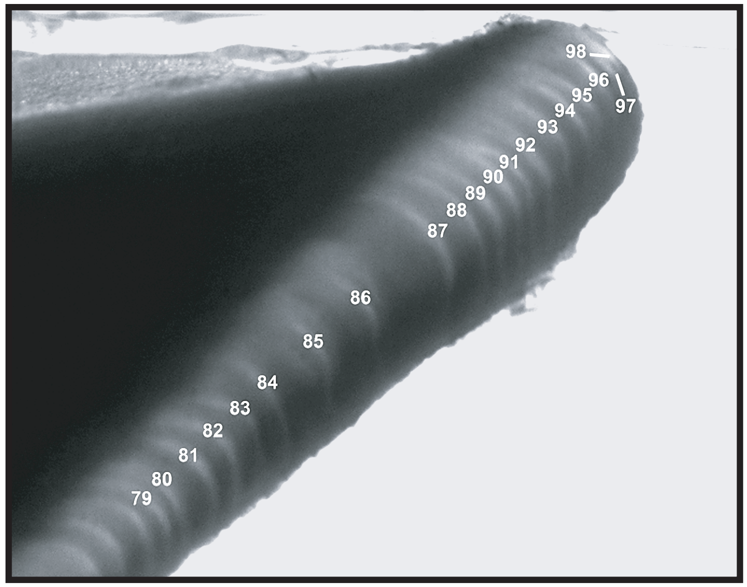

Figure 2

Enlargement of photomicrograph of a transverse vertebral section of a leopard shark (*Triakis semifasciata*) showing years (abbreviated in figure) that correspond to annuli distal to the tetracycline mark at year 1979 on the corpus calcareum.

Discussion

The tagging and recapture of fish whose calcified structures have been marked with the calcium-labeling fluorophor OTC offer a simple and conclusive method for establishing the timing of growth zone formation in these structures. Validating the timing of centrum or spine band formation is especially important for elasmobranchs as a group because, unlike teleosts, the assumption of annual deposition of band pairs has not been confirmed for many species and has been challenged for some. It has been suggested that two band pairs are deposited annually in the basking shark, *Cetorhinus maximus* (Parker and Stott, 1965) and the shortfin mako shark, *Isurus oxyrinchus* (Pratt and Casey, 1983), although validation is still pending for these species. Furthermore, Natanson (1984) and Natanson and Cailliet (1990), working with OTC-injected Pacific angel sharks, *Squatina californica*, found no correlation between centrum band deposition and any temporal cycle and concluded that bands were deposited in response to somatic growth. Nevertheless, elasmobranchs for which the deposition of one band pair per year has been established and validated with OTC (for at least selected size and age classes) include the thornback ray, *Raja clavata*, (Holden and Vince, 1973; Ryland and Ajayi, 1984); *R. microocellata* and *R. montagui* (Ryland and Ajayi, 1984); *Raja erinacea* (Natanson, 1993); *Bathyraja* sp. (Gallagher and Nolan, 1999); lemon shark, *Negaprion brevirostris* (Gruber and Stout, 1983; Brown and Gruber, 1988); spiny dogfish, *Squalus acanthias* (Beamish and McFarlane, 1985; Tucker 1985) leopard shark, *Triakis semifasciata* (Smith, 1984; Kusher et al., 1992); neonate sharpnose, *Rhizoprionodon terraenovae*, and sandbar, *C. plumbeus*, sharks (Branstetter 1987); and bonnethead shark, *Sphyrna tiburo* (Parsons 1993).

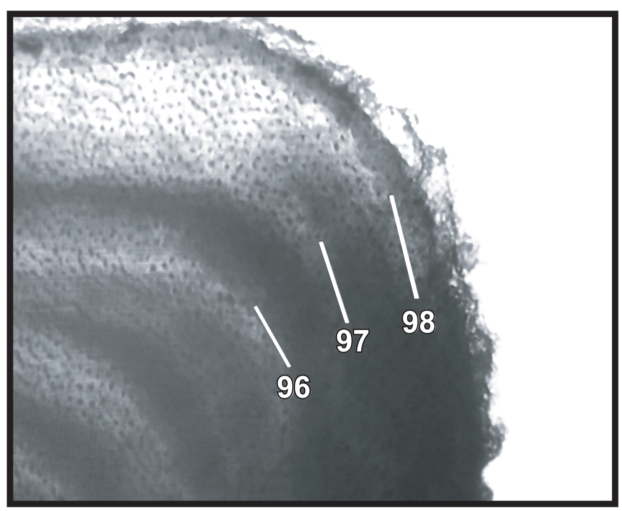

Figure 3

Enlarged view of the edge of the corpus calcareum of a transverse section of a leopard shark (*Triakis semifasciata*) vertebra, showing partially complete annuli at the centrum edge.

By demonstrating the persistence of OTC in the mineralized tissue of a cartilaginous fish at liberty for nearly two decades, our study confirms the long-term effectiveness of this age validation tool. The number of band pairs conformed to the number of years at liberty, although bands near the centrum periphery appeared in some sections to be absent or incompletely formed, even after careful embedding and sectioning. This emphasizes the importance of interpreting bands nearest the growing margin with extreme care, especially when examining band patterns in older, slow-growing sharks. For these fish, less precise preparation methods (such as examining whole centrum faces) are probably inadequate for interpreting narrow growth bands that form later in life. Additionally, in species like the leopard shark, the most recently deposited band pair may only become clearly defined or fully differentiated as new tissue after the accretion of subsequent bands. Thus, although most band pairs seem to be relatively well defined in this species (even in older specimens such as this one), the peripheral bands can be indistinct or incomplete. Beamish and McFarlane (2000), who recently reported on a sablefish (*Anoplopoma fimbria*) recaptured up to 20 years after being injected with OTC, also warned that the aging structures of long-lived fish require very careful preparation and interpretation.

In the vertebrae of the 1999 recaptured leopard shark, substantial centrum growth occurred in the late 1980s, e.g. the width of the 1987 growth zone (at 13 yr old) was over twice that of annual growth zones adjacent to it. We cannot explain why this period may have been so favorable for this shark over previous and subsequent years, but it does suggest that this species is capable of great interannual variability in growth, even at a relatively advanced age. Lenarz et al. (1995) described mild El Niño conditions off central California in 1987–88, although temperatures were less elevated than during other El Niño events, and only short periods of unusually high sea-level anomalies were observed. Moser et al. (2002) classified 1985–87 as a "normal" oceanographic period off California, between the warm 1982–83 El Niño episode and a cool-water 1988 La Niña episode.

OTC fluorescence is known to fade with exposure to UV light; therefore samples are traditionally stored in darkness and processed in dim light (Weber and Ridgeway 1962). The OTC mark in the leopard shark at liberty for 20 years was especially light-sensitive, disappearing after a few minutes exposure, much more quickly than tetracycline marks observed previously by one of the authors in earlier recaptures from this study. This may be due to diminishment of the phosphorescent properties of the chemical, combined with compression of the mark within the cartilage matrix over time. Therefore, when working with cartilaginous fishes (especially those at liberty for many years), it may be extremely important to obtain as many vertebrae as possible, to keep OTC-labeled centra away from light, to preplan microscope viewing sessions carefully, and to process and photograph samples quickly.

Previous studies have validated annual periodicity in leopard sharks up through age 17 (Smith 1984; Kusher et al. 1992). Beamish and McFarlane (1983) pointed out that band periodicity must be established for a full range of age classes to fully meet the requirement of age validation in fishes. This requirement is seldom met and has not yet been achieved for any elasmobranch. The OTC-labeled fish recaptured in 1999 measured 2 cm longer but was 8 years older than the oldest age-validated leopard shark of Kusher et al. (1992). This latter fish, also a female, measured 122 cm TL and was estimated to be 17 yr of age. It had been at liberty for 7.3 years—the longest duration recorded in that study. Results of the present study extend age validation for this species to fish at liberty for up to 19+ years, up to 124 cm TL, and up to 25 yr old. Longevity of this species has been estimated at 25–30 years (Smith, 1984); thus full validation for this species spanning ages 0 through 25 is now complete.

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