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BRIEF COMMUNICATION

Oxytetracycline age validation of an adult shortfin mako shark *Isurus oxyrinchus* after 6 years at liberty

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Running header: OXYTETRACYCLINE AGE VALIDATION OF *ISURUS OXYRINCHUS*

This study presents findings on an oxytetracycline injected adult male shortfin mako *Isurus oxyrinchus* recaptured in waters off of southern California after 6 years at liberty. During the period at liberty, the vertebral band-pair deposition rate was validated at one per year. This result indicates that from a time at or near sexual maturity, male *I. oxyrinchus* in the north-east Pacific Ocean exhibit a band-pair deposition rate of one band pair per year, while deposition rates for juveniles in the area have been validated at two band pairs per year.

Key words: band-pair periodicity; shifting deposition rate.

The age and growth of the shortfin mako *Isurus oxyrinchus* Rafinesque 1810 has been studied since the early 1980s (Cailliet & Bedford, 1983; Pratt & Casey, 1983; Francis & Duffy, 2005; Ardizzone *et al.*, 2006; Bishop *et al.*, 2006; Natanson *et al.*, 2006; Cerna & Licandeo, 2009; Semba *et al.*, 2009; Wells *et al.*, 2013; Doño *et al.*, 2015; Kai *et al.*, 2015). Several methods have been used in past studies including length-frequency analysis and tag-recapture growth, but the most common method for age determination has been counting alternating growth bands in vertebral centra. Most previous age and growth studies on *I. oxyrinchus* either concluded or assumed the vertebral band-pair deposition rate to be one band pair per year. Band-pair periodicity validation from bomb-radiocarbon chronologies (Ardizzone *et al.*, 2006) and a recaptured oxytetracycline-tagged *I. oxyrinchus* after over 1 year at liberty (Natanson *et al.*,

2006) support one band pair per year deposition rates in adults, but each of these studies recognized the possibility of biannual band-pair deposition for the first few years.

Wells *et al.* (2013) used oxytetracycline (OTC) to validate the deposition rate of band pairs on the vertebrae of 29 juvenile *I. oxyrinchus*, all d 200 cm fork length (L_F) at recapture, originally caught along the southern California coast. The results from the OTC validation, corroborated by growth rates calculated from tag-recapture growth and length-frequency data from commercial and research catch, showed that juvenile *I. oxyrinchus* in the north-east Pacific Ocean deposit two band pairs per year (biannual deposition rate) for at least the first 5 to 6 years of life. The validation of this deposition rate helps to explain why many length-at-age models apparently underestimate growth of juvenile *I. oxyrinchus* (Pratt & Casey, 1983; Bishop *et al.*, 2006; Natanson *et al.*, 2006; Kai *et al.*, 2015); without information from mature individuals, however, uncertainty of the deposition rate persists into adulthood.

With the recapture of an individual OTC-tagged adult male *I. oxyrinchus* in the waters off southern California after 6 years at liberty, it is possible to shed some light on the band-pair deposition periodicity at sizes above 200 cm L_F.

A mature male *I. oxyrinchus* was captured on a baited pelagic longline set for 4 h in the southern California Bight during a NOAA longline research cruise on 26 June 2008. The individual was measured (straight-line L_F), sexed, tagged in the dorsal musculature with a conventional spaghetti tag (Floy Tag and Manufacturing; www.floytag.com) and on the dorsal fin with a plastic roto tag specifying instructions for sampling vertebrae upon recapture (Dalton

ID Systems; www.Dalton.co.uk), and given an intraperitoneal injection of 11 ml of OTC (Agrimycin 200, Valley Vet; www.valleyvet.com), a dose rate of *c.* 25 mg kg^{-1} body mass (based on estimated length and mass conversions) before being released.

The same s *I. oxyrinchus* was recaptured on a baited pelagic longline set for 4 h in the southern California Bight during a NOAA longline research cruise on 1 July 2014. The shark was exhausted and in poor condition due to fighting while on the line, and upon confirmation that it had been previously tagged with OTC, it was euthanized. This *I. oxyrinchus* represents the first adult male injected with OTC and subsequently recaptured in the north-eastern Pacific Ocean after an extended period at liberty, offering the opportunity to directly assess the band-pair deposition rate of an adult *I. oxyrinchus* in an environment where a previous validation study on juveniles showed a pattern of biannual band-pair deposition.

Measurements of the shark were taken and vertebrae were excised from a region anterior to the dorsal fin, frozen, and transported back to the laboratory for processing. In the laboratory, thawed vertebrae were cleaned of excess tissue, sectioned using a low speed circular saw (Buehler Isomet; www.buehler.com), the OTC band was identified and marked under UV light, then the section was X-rayed. For more details on the methods of capture, tagging, or vertebrae processing used in this study, see Wells *et al.* (2013).

Digital images of the vertebrae X-rays were used to count and measure band pairs on a computer using ImagePro software (MediaCybernetics; www.mediacy.com). One translucent band (hypomineralized; appearing dark in X-ray) and one opaque band (hypermineralized;

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appearing light in X-ray) constituted a complete band pair. If a count ended in a terminal opaque band, a plus was added to the count to represent an incomplete band pair. Counts were made independently by two readers without prior knowledge of length, sex or time at liberty.

In order to identify where the tagged *I. oxyrinchus* fitted within the size range of males considered mature in prior studies, its size was compared with published male size-at-maturity estimates (Joung & Hsu, 2005; Conde-Moreno & Galván-Magaña, 2006; Semba *et al.*, 2011). Conversion equations from Joung & Hsu (2005) and Semba *et al.* (2011) were used to convert total length (L_T) and precaudal length (L_{PC}) to L_F, respectively.

Originally captured at 194 cm L_F, the animal was larger than the estimated size at 50% maturity for male *I. oxyrinchus* in the north Pacific Ocean [*c.* 172 cm L_F (Semba *et al.*, 2011), *c.* 188 cm L_F (Joung & Hsu, 2005), *c.* 161 cm L_F (Conde-Moreno & Galván-Magaña, 2006)], and also near the maximum size studied in Wells *et al.* (2013). After 2196 days at liberty (> 6 years), the animal was recaptured and measured 217 cm L_F, thereby growing 23 cm in 6 years with an average of 3.8 cm year^{-1}. Wells *et al.* (2013) estimated that juvenile *I. oxyrinchus* grow an average of 27–36 cm between their first and second summer and 20–29 cm in the following year. Continuously decreasing growth rate approaching an asymptote over time is a common pattern found across fish species including elasmobranchs. For *I. oxyrinchus* males, Doño *et al*. (2015) described growth phases with an inflection (slowing) in growth at the age at maturity.

A final band count along the corpus calcareum, made by two independent readers, indicated a total of 15+ band pairs, with the terminal edge of the vertebra displaying an incomplete opaque band (Fig. 1). The fluorescent OTC mark appeared within the opaque portion of the tenth band pair. Based on its size at first capture, this 10th band pair probably represents a band pair formed when the shark was at or near sexual maturity. The most recent validated age information for *I. oxyrinchus* < 200 cm L_F in the north-east Pacific Ocean would indicate that these 10 band pairs represent 5 years of growth (Wells *et al.*, 2013). Additionally, 5+ band pairs were visible distal to the OTC mark, indicating that in its 6 years post-tagging, this individual displayed an annual band-pair deposition rate. Based on these OTC validation studies, it can be inferred that male *I. oxyrinchus* experience rapid growth and biannual band-pair deposition as juveniles, followed by slowed growth and annual band-pair deposition as adults. The exact point at which this transition may occur is still uncertain, but it is probably after the first 5 years and near the age of sexual maturity. The age of this 217 cm L_F male *I. oxyrinchus* was estimated to be between 10 and 11 years.

Measurements from the distal edge of each growth band indicate that spacing was greater and more variable between band pairs prior to the OTC mark (1.01 ± 0.49 mm; mean ± S.D.), compared with band-pair spacing after the OTC mark (0.43 ± 0.20 mm; Table I). Previous ageing studies of lamnids have noted more regular and evenly spaced band pairs (Wells *et al.*, 2013) and a narrowing of band pairs near the periphery of the corpus calcareum (Francis *et al.*, 2007; Groeneveld *et al.*, 2014) in larger animals. The greater and more variable spacing of juvenile band pairs is potentially the result of rapid, yet inconsistent growth. With juvenile *I. oxyrinchus* committing significant resources to somatic growth, their growth rate (and hence the spacing of their band pairs) may be a product of their foraging success. More successful periods should result in more growth prior to band-pair completion, resulting in larger gaps between band pairs. Band-pair spacing may become more regular in adults as growth slows, perhaps indicating that resources are being allocated more evenly to other aspects of life, such as reproduction. Differences in diet (Preti *et al.*, 2012), movements (Wells *et al.*, 2013) and diving behaviour between adults and juveniles (Sepulveda *et al.*, 2004) may also contribute to dissimilarities in band-pair spacing. Importantly, however, other methods have indicated that band-pair deposition may change or even stop in advanced ages for some elasmobranch species (Andrews *et al.*, 2011; Hamady *et al.*, 2014; Natanson *et al.*, 2014).

The OTC results presented here validate male *I. oxyrinchus* vertebral band-pair deposition rates in the north-east Pacific Ocean up to *c.* age 11 years. When combined with studies from the Atlantic Ocean (off of the eastern U. S. and South Africa) (Ardizzone *et al.*, 2006; Natanson *et al.*, 2006), it appears that adult male *I. oxyrinchus* up to the age of 18 years deposit one vertebral band pair per year. Beyond 18 years, it is uncertain whether adult male *I. oxyrinchus* band-pair deposition rates remains consistent. Proper age determination and accurate growth models are important components of stock assessments and productivity analyses. A shift in band-pair deposition rates affects growth models (length at age) for male *I. oxyrinchus* in the north-east Pacific Ocean. Given uncertainty about deposition rates in female *I. oxyrinchus* in the north-east Pacific Ocean, and the potential for regional heterogeneity in growth, until further studies help resolve details across regions, sexes and sizes, multiple growth models for *I. oxyrinchus* may be necessary to capture the full range of uncertainty in parameters such as natural mortality rates, age at maturity, fecundity and longevity.

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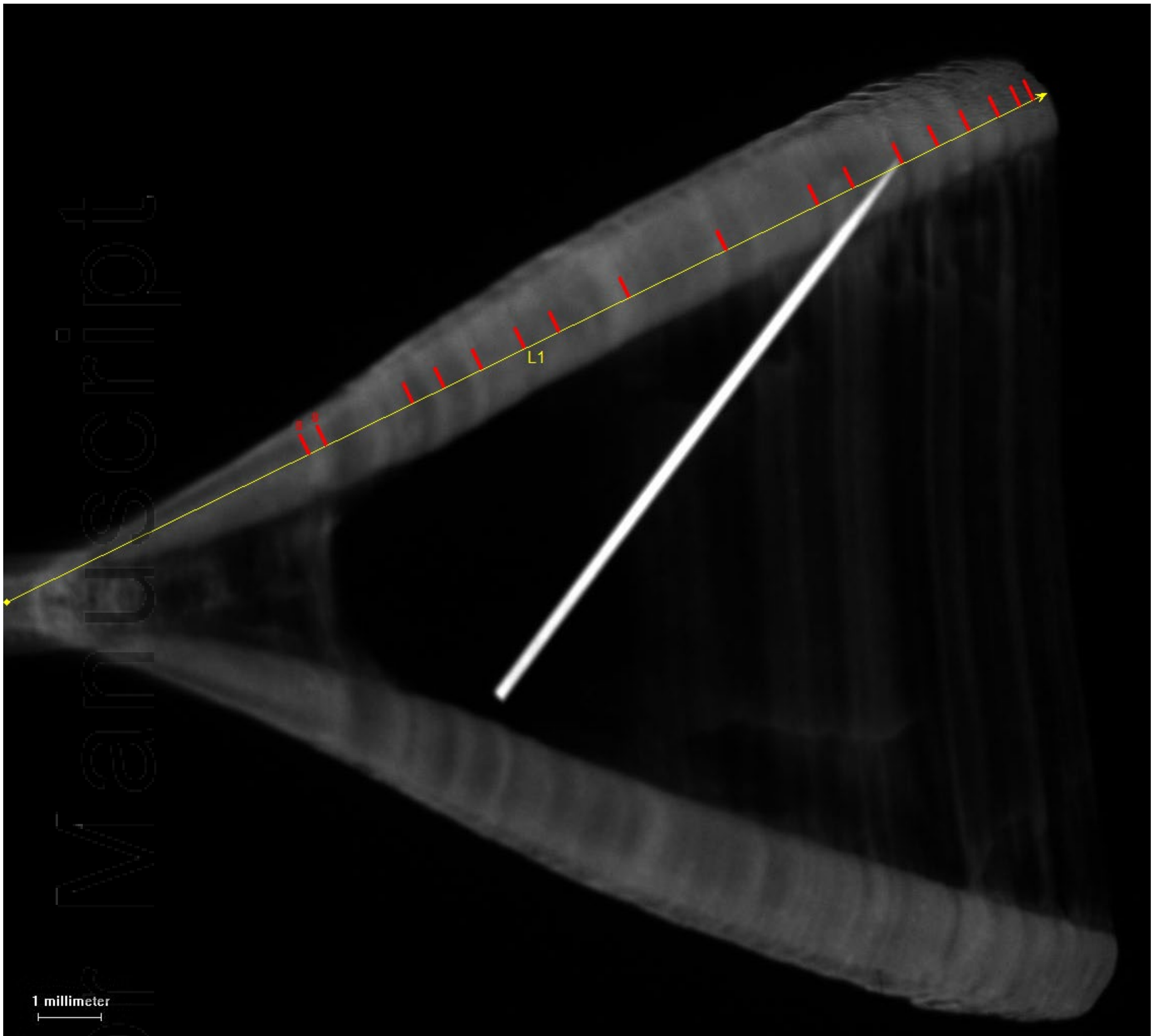

Figure 1

FIG. 1. X-ray image of a vertebral section from the recaptured *Isurus oxyrinchus*. Red marks indicate the distal edge of growth band pairs (excluding the first two red marks which indicate the proximal and distal edge of the birth band, which is opaque). A metal needle points to the location of the OTC mark on the vertebra.

TABLE I. Distances between band pairs and the estimated life stage based on published size-at-maturity studies for *Isurus oxyrinchus*. The first measurement is from the distal edge of the birth band to the distal edge of the first band pair

Band pair	Estimated age (years)	Distance to previous distal edge (mm)	Estimated stage
1	1	1.41	Juvenile
2	1	0.59	Juvenile
3	2	0.62	Juvenile
4	2	0.74	Juvenile
5	3	0.72	Juvenile
6	3	1.17	Juvenile
7	4	1.80	Juvenile
8	4	1.60	Juvenile
9	5	0.50	Juvenile–adult transition
10*	5	0.90	Juvenile–adult transition
11	6	0.70	Adult
12	7	0.50	Adult
13	8	0.60	Adult
14	9	0.30	Adult
15	10	0.30	Adult
15‡	10+	0.20	Adult

*, the band containing the OTC mark; ‡, an incomplete band pair with the final measurement made to the visible edge.