ISC/12/SHARKWG-1/01

Preliminary age validation of the blue shark (*Prionace glauca*) in the eastern Pacific Ocean

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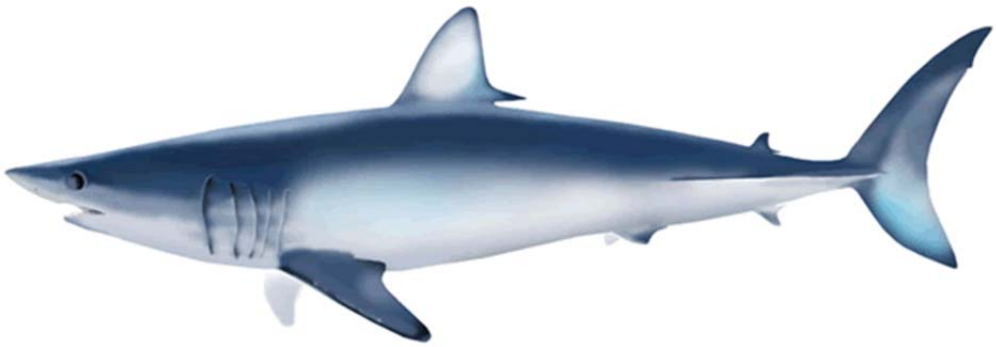

Working document submitted to the ISC Shark Working Group Workshop, 28 May – 4 June, 2012
National Research Institute of Far Seas Fisheries, Shizuoka, Japan

ABSTRACT

Accurate age and growth models are some of the most important biological parameters needed for stock assessment and fishery management. The blue shark (*Prionace glauca*) is subjected to one of the highest levels of fishery bycatch in the world and is the shark species caught in the greatest number in the California/Oregon drift gillnet fishery where most are discarded at sea due to a lack of market value. Despite their numerical importance, the stock status of blue shark in the North Pacific is uncertain. Assumptions regarding band pair deposition rates used for age and growth models are being made without validation studies in the Pacific Ocean. As such, the purpose of this study is to validate vertebral band counts of blue sharks tagged and recaptured in the eastern Pacific Ocean. Oxytetracycline (OTC) labeled vertebrae of 13 blue sharks have been obtained from tag-recapture activities and processed to determine timing of centrum growth band deposition. Several methodologies were used to examine blue shark vertebrae and digital images of the whole vertebrae centrum were determined to be the best. OTC tagging of the recaptured sharks occurred off southern California from 2007 to 2009, with time at liberty ranging from 22 to 473 days. For vertebrae samples used in this study, shark size at release ranged from 90 to 276 cm total length (TL). OTC marked vertebrae from at least 20 more sharks have been returned and will be processed to build upon this study. Results from band counts of vertebrae distal to OTC marks thus far indicate a single band pair (1 translucent and 1 opaque) is formed per year for blue sharks of the size range examined. These preliminary results corroborate annual deposition rates found in the only other OTC validation study for blue sharks and will aid in future blue shark age and growth studies in the Pacific Ocean.

INTRODUCTION

The blue shark, *Prionace glauca*, is the most abundant pelagic shark worldwide in tropical and subtropical seas, where it is found throughout oceanic and neritic waters (Pratt 1979, Nakano & Stevens 2008). Blue shark is also subjected to one of the highest levels of fishery bycatch in the world and is the shark species caught in the greatest number in the California/Oregon drift gillnet fishery targeting swordfish where most blue sharks are discarded at sea due to a lack of market value (Hanan et al. 1993). The blue shark to target swordfish catch ratio is approximately 1:1 and the majority of sharks caught are discarded dead (Holts et al. 1998). Because of their vulnerable life history characteristics relative to co-occurring teleost fish and their role as top predators, ,sound management of blue sharks is important from both a single-species and ecosystem-based perspective.

Accurate size-at-age determinations are necessary for both stock assessment and management of sharks because they form the basis for calculations of growth and mortality rates, age-at-maturity, age-at-recruitment, and estimates of longevity. Blue shark growth rates are moderately fast with males and females reaching sexual maturity between 4-6 and 5-7 years, respectively (Nakano 1994). Age and growth studies of blue sharks in the North Pacific and North Atlantic have been fairly well documented with size-at-maturity of about 200 cm total length (TL) (~170 cm fork length, FL) for both sexes in the North Pacific (Suda 1953, Nakano et al. 1985). In the western Atlantic, 50% sexual maturity of male blue sharks averaged 218 cm TL (~180 cm FL) and females were fully mature by 221 cm TL (~185 cm FL) (Pratt 1979). Maximum longevity of blue sharks is approximately 20 years with a maximum size of 383 cm TL (320 cm FL) reported from the northwest Atlantic Ocean (Bigelow and Schroeder 1953).

A suite of band enhancement techniques have been used in elasmobranch vertebrae studies including digital images of vertebral sections (Natanson et al. 2002, MacNeil & Campana 2002), x-ray radiography (Cailliet et al. 1983, Liu et al. 1998), and staining of vertebrae (Stevens 1975). Validation of annual band pair deposition for blue sharks up to four years of age was performed using vertebrae from two oxytetracycline (OTC)-injected sharks in the North Atlantic (Skomal & Natanson 2003). Results suggested an annual spring deposition of growth zones within the vertebrae and used supporting migratory and life history information of this species as a plausible explanation of annulus formation. For blue sharks, several researchers have used silver nitrate staining on vertebrae (Cailliet & Bedford 1983, Nakano 1994, Blanco-Parra et al. 2008), haematoxylin-eosin staining (Tanaka et al. 1990), and sectioned vertebrae bow ties imaged under light microscopy (Skomal & Natanson 2003). Given the diversity of techniques used, a secondary objective of this study was to explore several techniques for processing blue shark vertebrae.

The primary objective of this study was to validate the periodicity of band pair deposition in blue shark vertebrae collected in the eastern Pacific Ocean. Numerous studies examining age and growth of blue sharks have been performed in the Pacific Ocean; however, no studies have validated the deposition of band pairs. Chemical marking methods are some of the most robust age validation techniques available (Campana 2001, Goldman 2005). Consequently, blue sharks were OTC-injected and recovered vertebrae were examined to determine the banding pattern deposited distal to the OTC mark during the known time at liberty.

MATERIALS AND METHODS

Sharks for tagging and OTC injection were captured in the Southern California Bight (SCB) using baited pelagic longlines. Leaders were unsnapped from the main line and sharks

guided into a semi-submerged metal tagging cradle at the stern of the vessel. The cradle was then raised to facilitate tagging, measuring, and OTC injection, while the eyes of the shark were covered with a wet chamois cloth, and a saltwater ventilation hose continuously pumped water over the shark's gills. Once injected, each shark was tagged on the dorsal fin with a plastic rototag labeled with contact and reward information in English and Spanish, with instructions to measure the fish and save the vertebrae. Most sharks were also double-tagged with a spaghetti tag placed in the dorsal musculature beneath the first dorsal fin. At tagging, each shark was sexed and measured (straight line FL or TL) to the nearest centimeter using a stationary measuring device fitted to the shark tagging cradle. FL was converted to total length (TL) if necessary for purposes of this paper using Kohler et al. (1995): TL = [FL-1.39]/0.8313). Sharks were given an intraperitoneal injection of OTC at a dose rate of 25 mg/kg of body weight and then released.

Oxytetracycline-marked vertebrae were obtained from blue shark recaptured on research cruises and commercial and recreational fishing vessels between 2007 and 2010 along the coast of California and Baja California (Figure 1). Samples were stored frozen until processed, and kept from light and excessive UV exposure to preserve the OTC mark. Widest diameter vertebral centra in a given sample were chosen for processing. Whole centra were separated, cleaned of excess tissue, rinsed and air-dried. To elucidate the vertebral bands, we chose to compare several techniques in order to determine the most suitable method for the OTC-marked blue shark vertebrae. These included the high frequency X-radiography technique of Cailliet and Bedford (1983) and Cailliet et al. (1983), whole centrum faces and sectioned vertebrae bow ties viewed under a light microscope (Skomal & Natanson 2003), and Alizarin red staining. For staining experiments, vertebrae were decalcified using RDO (ranging from 15-30 minutes), then

bowtie sections were cut (sagittal slices 80-140 µm) using a microtome and stained using Alizarin red solution. Some samples were not decalcified (to preserve OTC mark) and cut to 0.1-0.3 mm using an Isomet low speed saw. In all techniques, ultra-violet light was used to fluoresce the OTC mark on the vertebrae. Digital photographs of whole centrum faces of vertebrae were determined to provide the best overall image quality for counting band pairs and were the primary technique for this study, similar to methodology used by MacNeil & Campana (2002). Samples were photographed using a Leica Z16 APO dissecting microscope with sub stage illumination and a digital camera.

Band pairs were counted from digital images of centrum faces on a computer screen. We referred to the original vertebrae under the microscope if more detail was desired. As in Bishop et al. (2004, 2006), counts excluded the birth band, which represents age zero. Alternating pairs of translucent (bright, broad=fast growth period) and opaque (dark, narrow=slow growth period) bands were assumed to represent one complete 'growth increment' or 'band pair' (terms we use synonymously). Two separate band counts were made: 1) total band pairs, or half the number of bands distal to the presumed birth band, and 2) band pairs distal to the OTC mark. Increment counting for the former began at the distal edge of the first translucent zone beyond the birth band, and for the latter, at the distal edge of the first translucent zone beyond the OTC mark.

Each sample was read independently by two readers. Bands were blind counted without knowledge of the fish length, sex, or time at liberty. Readers consulted with each other on criteria for counts prior to readings. Samples for which there was any disagreement were counted a second time and counts with similar readings between readers were deemed final. A least-squares linear regression analysis was performed and the null hypothesis that the slope of the relationship between the number of bands and time was equal to one (a situation occurring if

one opaque and one translucent band were deposited each year) was tested using a two-tailed t-test (Kusher et al., 1992).

RESULTS AND DISCUSSION

Preliminary results suggest a single band pair (1 opaque and 1 translucent) is laid down per year for blue sharks in the eastern Pacific Ocean. Of the 13 blue shark vertebrae examined, all blue sharks (n=5) at large greater than 200 days had one complete band pair post OTC (Table 1). Two sharks at large for 121-177 days had half the band pair, while all other sharks at large less than 90 days had very little growth to discern any band deposition post OTC (Table 1). All sharks were tagged in summer which coincides with the fast growth period (translucent, bright area in Figure 2). Consequently, sharks with a full band pair showed an OTC mark in the middle of the translucent zone, followed by an opaque (dark, slow growth) band and an additional translucent zone (Figure 2). Unfortunately, no sharks were at large long enough to observe a following opaque zone beyond this second translucent zone. Preliminary findings support formation of the opaque (slow growth) band around late fall (November) through winter months (December through March). These results are preliminary and limited to blue sharks tagged in the Southern California Bight study area that have been at liberty for just over a year or less. Nevertheless, they are consistent with the annual band pair deposition rate found for two OTC marked blue sharks from the North Atlantic (Skomal & Natanson 2003) and by bomb radiocarbon dating of two blue sharks in the Indian Ocean (Romanov & Campana 2011). Vertebrae of at least 20 more OTC marked blue sharks have been returned and will be processed to complete this study.

Though not the primary purpose of this study, size at age estimates were similar to previous studies examining age and growth of blue sharks in the North Pacific and North

Atlantic. Age-1 blue sharks ranged from 96 to 119 cm TL, age-2 ranged from 109 to 174 cm TL, and age-3 from 161 to 180 cm TL (Figure 3). The average size range predicted by von Bertalanffy growth models of this species ranges from 73-115 cm TL for age-1, 106-150 cm TL for age-2, and 131-180 cm TL for age-3 (Cailliet et al. 1983, Nakano 1994, Tanaka et al. 1990, Skomal & Natanson 2003, Blanco-Parra et al. 2008) (Figure 4). The two largest male blue sharks recaptured were 194 and 281 cm TL with estimated ages of five and nine years, respectively. Similarly, Nakano (1994) estimated a mean size of 201 cm TL for male blue sharks by age-5 and 275 cm TL by age-9 (Figure 4). Nevertheless, caution should be used when interpreting our absolute size at age results since six of the 13 recapture lengths were estimated.

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Table 1. Summary table of blue shark OTC-labeled vertebrae samples organized by time at liberty. Including tag and recapture dates and fish lengths, time at liberty, sex, and average number of band pairs (based on two independent readers) both after OTC and birth band.

Fish ID	Tag date	Recapture date	Time at liberty (days)	Sex	Tagging length (cm TL)	Recapture length (cm TL)	Total number of band pairs after OTC	Total number of band pairs after birth band
1.A039396	7/18/2007	11/2/2008	473	M	129	*174	1	2
2.A039514	6/17/2008	9/13/2009	453	M	133	157	1	2
3.A039427	6/22/2008	7/17/2009	390	M	129	180	1	3
4.A039864	6/20/2008	4/8/2009	292	M	90	*117	1	1
5.A040276	8/6/2009	3/6/2010	212	M	98	128	1	2
6.A039924	8/2/2007	1/26/2008	177	M	163	194	0.5	5
7.A039322	7/17/2007	11/15/2007	121	M	276	281	0.5	9
8.A040248	8/4/2009	11/1/2009	89	F	105	*114	0	2
9.A038861	7/30/2009	10/9/2009	71	F	154	*161	0	3
10.A039422	6/22/2008	8/8/2008	47	M	98	107	0	1
11.A040750	8/17/2009	9/29/2009	43	F	91	*96	0	1
12.A040824	8/25/2009	10/5/2009	41	M	104	*109	0	2
13.A039438	6/23/2008	7/15/2008	22	M	116	119	0	1

*indicates lengths were estimated. Note: all lengths were converted from FL to TL

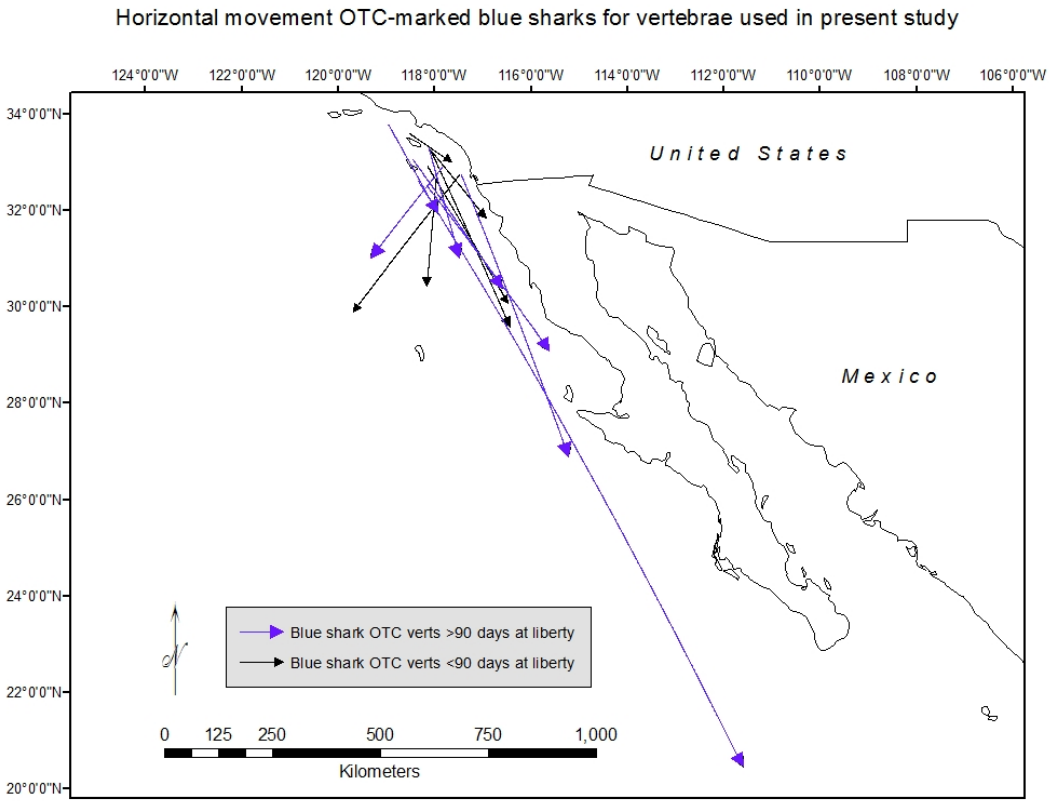

Figure 1. Map of study area with tag and recapture locations (triangles) for recaptured OTC-injected blue sharks (n=13) in the Southern California Bight during the study period. Purple and black arrows represent sharks at large greater and less than 90 days, respectively.

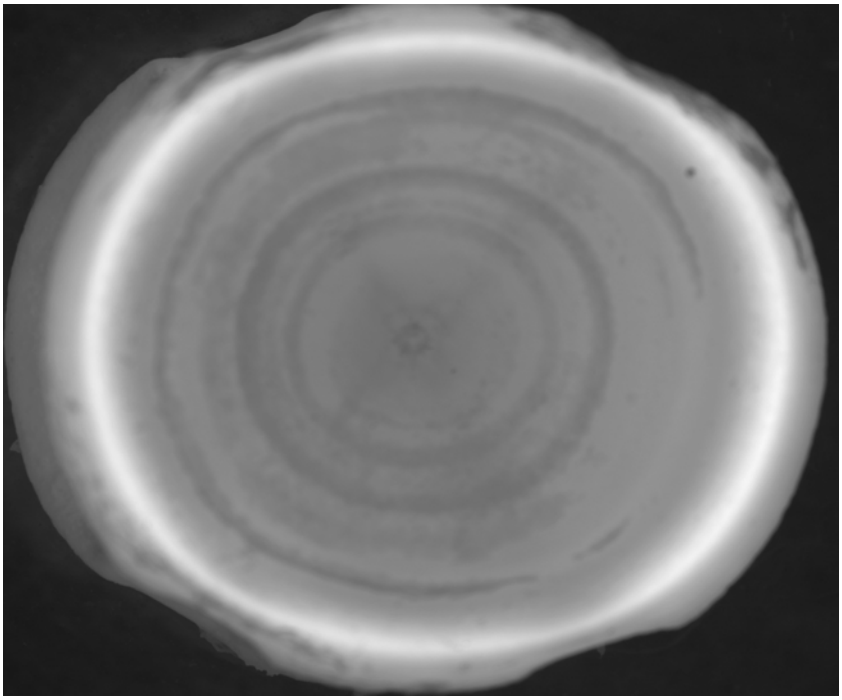

Figure 2. Whole vertebrae image showing band pair progression of an OTC-labeled blue shark. Bright ring shows the OTC fluorescence under ultraviolet light.

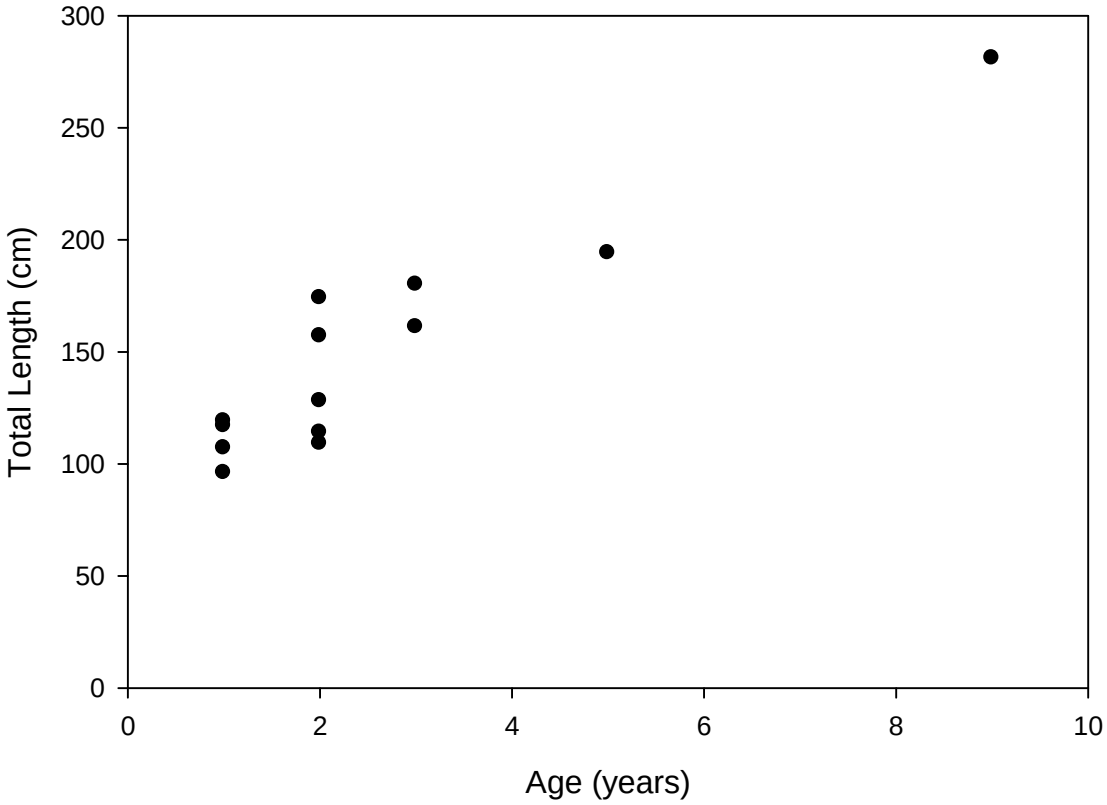

Figure 3. Preliminary size-at-age estimates of blue sharks from this study assuming annual band pair deposition rate.

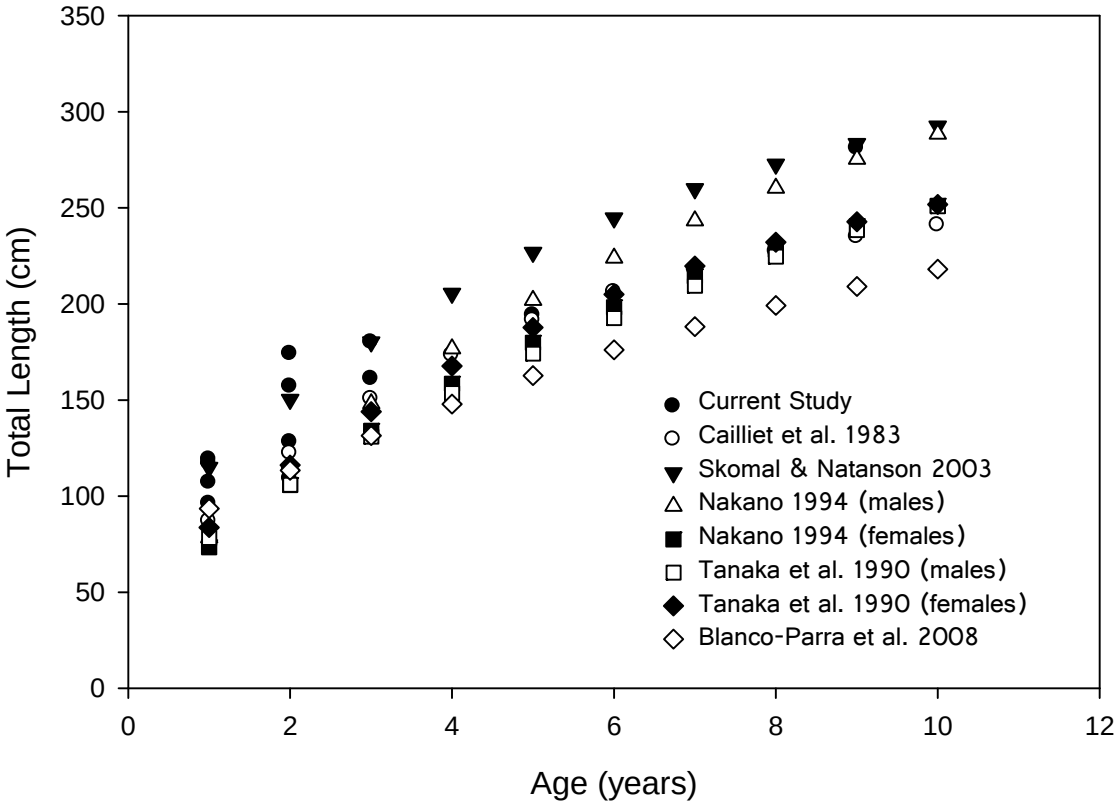

Figure 4. Size-at-age estimates of blue sharks from this study compared to other studies in the North Pacific and one North Atlantic study (Skomal & Natanson 2003), assuming annual band pair deposition rate.