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Insights into vertebral band pair deposition rate in the juvenile common thresher shark (*Alopias vulpinus*) in the northeastern Pacific Ocean

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Abstract

Validation of band pair deposition rates in elasmobranch vertebrae is essential for accurate age estimation using band pair counting techniques. We present a validation study of the vertebral band pair deposition rate for juvenile common thresher sharks *Alopias vulpinus* in the northeastern Pacific Ocean (NEPO) using tag and recapture with oxytetracycline (OTC) injection. A total of 14 juvenile *A. vulpinus* marked with OTC from 1998 through 2013 were recaptured with times at liberty ranging from 1.08 to 3.81 years with an average of 2.14 years (±0.97 years standard deviation, SD). Shark size ranged from 80 to 128 cm fork length (LF) at the time of OTC injection and from 112 to 168 cm LF for those measured at recapture. The slopes of the relationships between band pairs post OTC and years at liberty for each reader ranged from 0.84 to 0.95, slightly lower than the 1.0 slope expected from annual band pair formation. These findings preliminarily support previous age and growth assumptions based on a one band pair per year deposition rate. However, high variation in band pair deposition rates between samples, coupled with regression slopes falling just under one band pair per year, indicates that further investigation is needed to refine band pair deposition rate estimates.

KEYWORDS

age validation, *Alopias vulpinus*, growth, oxytetracycline (OTC), tagging, thresher shark, vertebrae

1 | INTRODUCTION

Sustainable management of fishing activity on exploited shark populations requires accurate life-history information. Stock assessment models that incorporate age, growth, reproduction, and movement are essential for understanding populations (Cortés, 2008). Quantitative assessments commonly rely on length or weight data of individuals caught. The quality of growth, longevity, age at maturity, and mortality rate estimates reflect the accuracy of biometric measurements and calculated size-at-age relationship estimates (Campana, 2001; Pardo et al., 2013). Validation studies are foundational to the accurate interpretation of age estimates that rely on band pair counts. This includes average age at first maturity, which is among the most influential parameters in determining shark population resilience to harvest, and may indicate rebound potential from overexploitation (Cortés, 2002; Smith et al., 1998). Validation can be achieved using a variety of methods such as mark-recapture of chemically tagged sharks, bomb radiocarbon analysis, release of known age marked or tagged sharks, and captive rearing (Cailliet et al., 2006). Each method presents its own set of advantages and challenges, and obtaining size-at-age relationships remains difficult.

A common technique for estimating age in elasmobranchs is counting band pairs formed in vertebral centra. These band pairs are formed by cartilage matrices of contrasting calcification densities. A band pair consists of a more calcified (hypermineralized) band and a less calcified (hypomineralized) band, in either order, deposited adjacent to one another, with deposition occurring distal to the centrum focus (Cailliet et al., 1983; Officer et al., 1996). The rate at which band pairs are deposited within the vertebrae (band pair deposition rate) in sharks can vary within and across species, geographic locations, and throughout ontogeny (Kinney et al., 2016; Natanson et al., 2016; Wells et al., 2013).

The common thresher shark *Alopias vulpinus* (Bonnaterre 1788) occurs in epipelagic neritic and oceanic waters in the Atlantic, Pacific, and Indian Oceans; and the Mediterranean Sea (Compagno, 2001). With relatively high trophic positions and specialized diets (Estrada et al., 2003; Preti et al., 2012), *A. vulpinus* play an important role in the ecosystem and may exert strong top-down effects on prey populations compared to more generalist species (Young et al., 2016).

In the northeastern Pacific Ocean (NEPO), the species is predominantly found in the U.S. and Mexico exclusive economic zones, migrating north and shoreward in warmer months (Moreno et al., 1989), and south during cooler months (Cartamil et al., 2010; Cartamil et al., 2011; Gonzalez, 2008; Hanan et al., 1993; O'Brien & Sunada, 1994). The Southern California Bight (SCB) provides important habitat for *A. vulpinus* <120 cm fork length (LF) close to shore (Cartamil et al., 2010; Cartamil et al., 2016), whereas *A. vulpinus* ≥120 cm LF are more frequently observed in the offshore waters of the California Current Large Marine Ecosystem (Cartamil et al., 2010; Cartamil et al., 2016; Pacific Fisheries Management Council, 2003). The common thresher shark is listed as a Species of Least Concern in the North Pacific, Critically Endangered in the North Atlantic, with a Global Status of Vulnerable by the IUCN (Rigby et al., 2022). The population status of *A. vulpinus* in the NEPO is currently not overfished or subject to overfishing (Teo et al., 2018). However, as top-level predators faced with climate-driven fluctuations in prey and potential bottom-up effects (Kaplan et al., 2017), fishery monitoring and regular population assessments are critical.

A. vulpinus of all sizes are subjected to fishing pressures within Mexico and the United States (Cartamil et al., 2011; Cartamil et al., 2016; Escobedo-Olvera, 2009; Gonzalez, 2008; Hanan et al., 1993; O'Brien & Sunada, 1994; Santana-Morales et al., 2020). After a decline in NEPO *A. vulpinus* numbers in the 1990s, the population took longer to recover than anticipated (Pacific Fisheries Management Council, 2003). Cortés (2008) later observed that elasticity and resiliency estimates based on the available growth data at the time were inconsistent with the slow recovery. Updated growth data published by Smith et al. (2008) produced lower productivity estimates more in line with the population growth trajectories. The Pacific Fisheries Management Council (PFMC) lists data gaps in age and growth rates, along with maturity and reproductive schedules for *A. vulpinus* among its most important research priorities (Pacific Fisheries Management Council, 2018).

Validation of band pair deposition rates for *A. vulpinus* in the region is needed to reduce uncertainty and improve accuracy in growth and resiliency analyses (Cortés, 2008), accuracy assessment of existing growth curves (Cailliet et al., 1983; Smith et al., 2008; Teo et al., 2018), and management strategy recommendations. The purpose of the current study is to present insight and current findings on validation of the vertebral band pair deposition rate for juvenile *A. vulpinus* in the NEPO.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The care and use of animals in this study complied with U.S. animal welfare laws, guidelines, and policies as approved by the National Oceanic and Atmospheric Administration (NOAA) Southwest Fisheries Science Center Institutional Animal Care and Use Committee. Animals impacted during tagging operations were handled as humanely as possible by field researchers who made efforts to minimize time out of water, and reduce and mitigate pain and distress of captured individuals throughout the process. Recapture data and biologic samples were obtained opportunistically through cooperation with fishers. NOAA engages in outreach and education with fishing communities. Continued research, outreach, and education with an emphasis on best fishing practices informed by perception of pain, nociception, and sentience may further reduce and mitigate pain and distress incurred by fish with whom humans interact.

2.2 | Tagging

A. vulpinus were captured on fishery independent surveys conducted by the California Department of Fish and Wildlife and NOAA Southwest Fisheries Science Center. Surveys began in 1994 and continued most years through 2017 (Runcie et al., 2016; Teo et al., 2018). Upon capture via longline, *A. vulpinus* were brought onboard the survey vessel using a cradle and immediately ventilated with a flow-through seawater hose fit with a PVC mouthpiece. All sharks were measured in straight-line distance from the snout tip to the fork in the caudal fin (fork length, LF: ±1 cm). Total length (LT: ±1 cm) was measured as a straight-line distance from the snout tip to the distal tip of the caudal fin, naturally extended, and alternate length (AL: ±1 cm) measured as the straight-line distance between the anterior origin (leading edge) of the first and second dorsal fins. An intraperitoneal injection of OTC was administered to each shark at a dose of 25 mg kg^{-1} body weight estimated based on length, and the dorsal fin tagged with a Rototag[1] (Dalton Tags, Newton, UK) indicating sampling instructions in English and Spanish, a contact address, and unique identifier (Wells

[1]Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

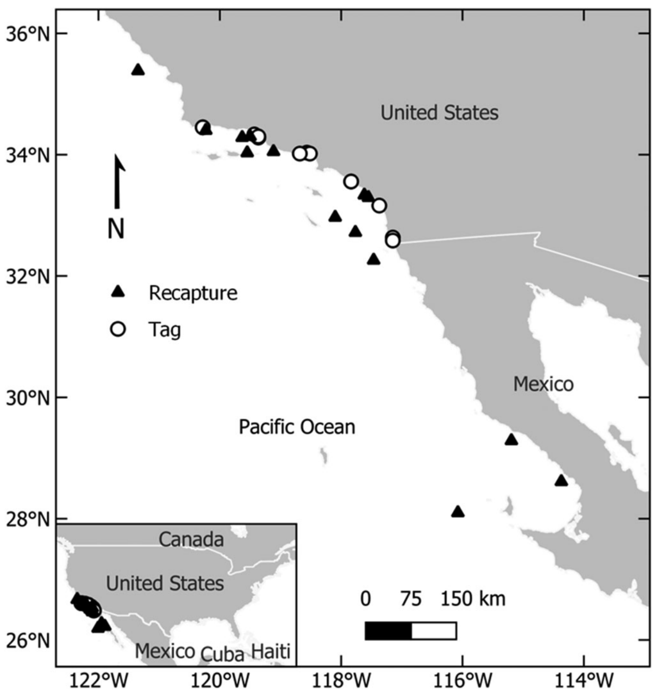

FIGURE 1 Tag and recapture locations for *Alopias vulpinus* whose vertebrae were used in this study ($n = 14$), Southern California Bight.

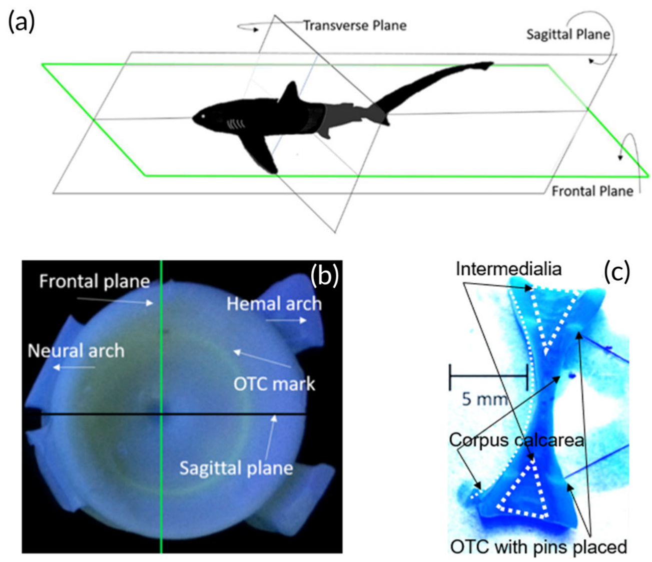

FIGURE 2 Vertebral preparation: (a) standard anatomical planes of a fish; (b) oxytetracycline (OTC) mark fluorescing under UV light; section is cut along the green line (frontal plane); and (c) pin placement with UV light. Image A is adapted from an image in Wilson et al. (1983).

et al., 2013). A conventional tag was placed in the dorsal musculature of each shark before release.

2.3 | Recapture

From 1998 to 2015, 1575 *A. vulpinus* (792 males, 769 females, and 14 of unknown sex) were injected with OTC, measured, and tagged. A total of 84 OTC-tagged *A. vulpinus* were recaptured between 1998 and 2015 (Figure 1; Supplementary Figures S1–S4). Samples from 61 OTC-marked *A. vulpinus* were returned. We conducted outreach to maximize sample return rates of tagged *A. vulpinus* including distribution of tagging programme information, and an award was offered for the return of vertebrae from recaptured tagged *A. vulpinus*. Participants were asked to collect LF, geographic data, and multiple vertebrae from just anterior to the caudal fin upon recapture. To maximize fisher participation and increase sample consistency, caudal vertebrae were requested; sampling from this region of the spinal column is easiest to access and least likely to impact market value.

However, as the exact location of sampled vertebrae could not be verified, we assumed that differences in band pair formation along the vertebral column were insignificant based on previous studies (Gervelis & Natanson, 2013; Natanson et al., 2018).

For the purposes of this study, only vertebrae from thresher sharks who had been at liberty for 1 year or more with readable OTC marks were included. Vertebral samples from a total of 14 *A. vulpinus* qualified for this study. These vertebrae were returned by nine fishers and researchers, with nine usable recapture lengths reported by eight participants.

2.4 | *A. vulpinus* length

Lengths reported for recaptured sharks were not always in LF. Thus, we used length measurements from observer data and shark research surveys to derive the following relationships and convert the lengths of recaptured sharks to LF (Data S1) where all lengths are in centimeter:

$$LF = 2.373\,(\mathrm{AL}) + 16.1, r^2 = 0.923, n = 3148$$

$$LF = 0.5311\,(LT) - 0.7464, r^2 = 0.972, n = 1056$$

Lengths at recapture for sharks in this study include directly measured, estimated, and converted lengths.

2.5 | Vertebral sample preparation

Returned vertebral samples were stored frozen until processed. Samples were protected from light to preserve the OTC mark between all handling steps. Excess flesh was gently removed from the vertebrae, and a 365 nm UV light was used to verify the existence of an OTC mark on each vertebral sample. Only those showing a distinct OTC mark were further processed. Each vertebra was submerged in water with detergent; a slow boil was used to separate the flesh from the centrum, with final cleaning by hand to ensure the corpus calcarea remained intact.

Cross-sections were prepared for imaging using methods based on those of Wells et al. (2013). Vertebral cross-sections cut along the frontal plane (intersecting and perpendicular to the sagittal plane, Figure 2a,b) were X-rayed. Two cuts were made, one on

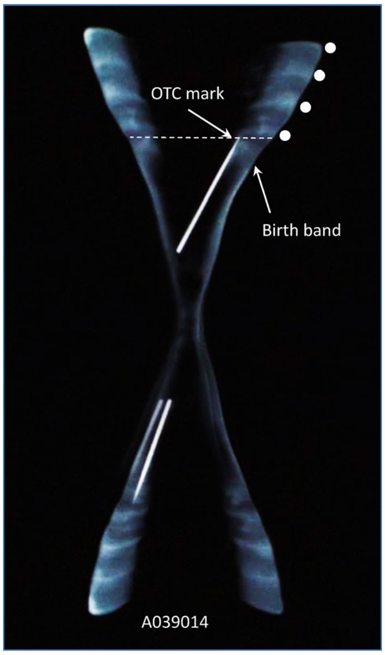

FIGURE 3 An X-ray image of a section showing band pair progression of an oxytetracycline (OTC)-marked *Alopias vulpinus* recaptured in the northeastern Pacific Ocean. Translucent cartilage (dark bands on image) alternated with more-calcified cartilage (appearing light on image). Opaque, hypermineralized bands are marked with a solid dot; 4 band pairs post birth band and 3.5 band pairs post-OTC. The OTC mark and birth band are labeled. Tagged *A. vulpinus* A039014 was at liberty 3.81 years, measured 80 cm fork length (LF) at the time of tagging, and 140 cm LF at recapture.

either side of the focus to create a section using a Buehler IsoMet saw (Uzwil, Switzerland). The section was then mounted on cardstock with super glue. Under UV light, stainless steel pins were positioned and glued into place to indicate the location of the OTC mark. A Leica dissecting microscope (Wetzlar, Germany), Olympus camera, and cellSens software (Tokyo, Japan) were used to create digital images of the X-rays. X-ray images showed contrast between bands. Translucent (hypomineralized) bands appear dark in standard (negative) X-ray images, and opaque (hypermineralized) bands appear light in standard (negative) X-ray images. After experimenting with different section thicknesses and exposures, we found c. 1-mm-thick sections, placed 6–10 cm from the X-ray head, for 15–25 s at 5 mA and 35 kV, using Kodak Industrex M and M100 film (Readypack II; Eastman Kodak Co., Rochester, NY, USA) produced the best image clarity and elucidation of banding patterns.

To determine the most effective technique for the preparation of *A. vulpinus* vertebrae, we compared various techniques to elucidate bands before choosing the methods described earlier. Our comparison included the illumination of whole centra faces using transmitted light and captured via digital photography, reflected light with digital photography, examination of 0.4–0.6-mm-thick sections under a microscope, and the staining of sections with Alizarin Red. Digital X-ray and X-ray with film techniques were also explored. We found imaging sections with hard X-rays using a General Electric (Fairfield, CT, USA) Mobile 100–15 X-ray unit was the most effective method for maximizing the visible contrast between bands.

TABLE 1 Summary table of oxytetracycline (OTC) marked vertebrae samples from *Alopias vulpinus* tagged and recaptured from 1998 to 2013 and at liberty ≥1 year in the northeastern Pacific Ocean.

Fish ID	Length at tagging (cm LF)	Length at recapture (cm LF)	Sex	Tag date	Recapture date	Years at liberty	BP POTC	BP PBB
A039014	80	140[a]	M	9/7/2006	6/27/2010	3.81	3.5	4
A039063	85	143[b]	F	9/9/2006	6/25/2010	3.79	4.50 ± 0.41	4.83 ± 0.47
A079061	101	115	F	9/13/2009	2/20/2013	3.44	2	3
A039565	113	165[a]	F	9/5/2007	10/13/2010	3.11	2.17 ± 0.47	2.83 ± 0.94
A039019	83	NL[c]	F	9/7/2006	4/25/2009	2.63	2	2
A079055	128	168[a]	F	9/13/2009	7/11/2011	1.82	2.5	5
A039543	105	NL[c]	F	9/7/2007	6/30/2009	1.81	1.17 ± 0.24	2.17 ± 0.24
A039631	98	NL[c]	M	9/15/2007	6/2/2009	1.72	1.67 ± 0.24	3.5
A040636	114	132[a]	F	9/18/2008	4/13/2010	1.57	1.5	2
A038148	108	129[a,b]	M	9/13/2006	1/20/2008	1.35	1.33 ± 0.24	2.33 ± 0.47
A038470	85	128	M	8/16/2004	12/18/2005	1.34	0.83 ± 0.24	1.83 ± 0.24
A039569	98	NL[c]	M	9/5/2007	12/2/2008	1.24	1.67 ± 0.24	2.5
A032625	112	NL[c]	F	7/12/1998	10/2/1999	1.22	1	2.5
A039069	101	112	M	9/9/2006	10/10/2007	1.08	0.83 ± 0.24	2.17 ± 0.47

Note: Samples are sorted by decreasing time at liberty. The number of band pairs (BP) distal to the OTC mark and birth band represent agreement among all three readers or the average across three readers shown with standard deviation ($n = 6$ and $n = 8$, respectively). POTC, post OTC.
[a]Measurement was converted from inches to centimeters.
[b]Fork length (LF) was converted from total length or alternate length using the length regressions provided in the current study.
[c]Either no length estimate provided by recapture party or length provided was unreliable.

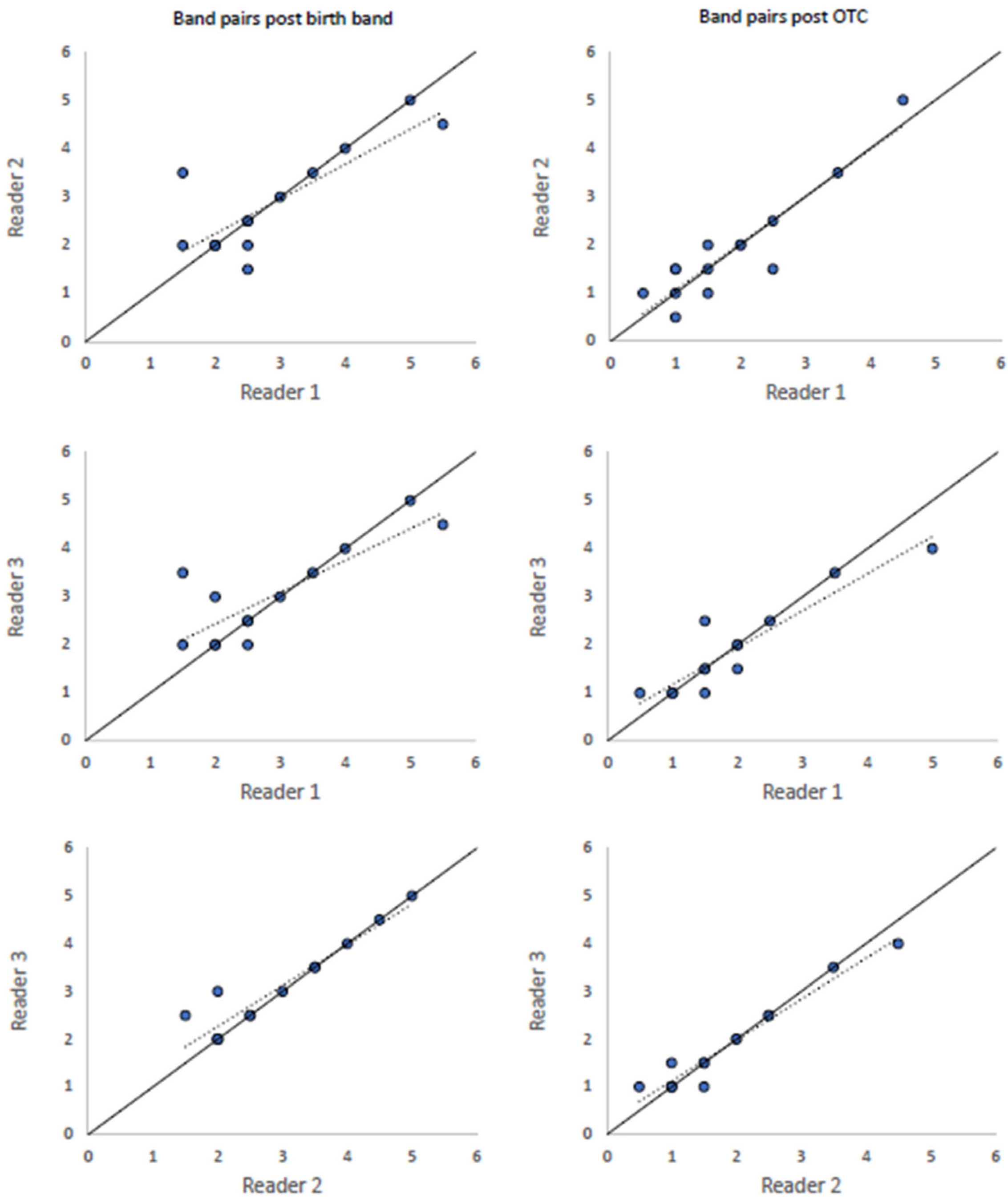

FIGURE 4 Age-bias plots for all oxytetracycline (OTC)-marked *Alopias vulpinus* vertebrae to determine the number of band pairs distal to the birth band (left column), and number of band pairs distal to the OTC mark (right column). Readers 1, 2, and 3 read all sample vertebrae without knowledge of fish ID or time at liberty.

2.6 | Age validation-band pair counts

Three independent readers each provided band pair counts for the complete collection of vertebral sections using digital images of X-rays. If more visual detail was needed, the readers referred to the original X-rays. Readers primarily used marks appearing on the corpus calcarea in X-rays to distinguish bands. Patterns in images of the intermedialia were used to corroborate existence of a questionable band when section morphology and image quality allowed (Figures 2c and 3). To ensure consistent counting methods, each of the three readers received a blank counting worksheet, a written counting protocol, and an illustrated instructional guide. All readers reviewed the counting protocol together using non-study vertebrae from a training collection before independently reading study samples.

The birth band (BB) was identified as the first distinguishable hypermineralized band, positioned where a subtle change in the centrum angle occurred (Casey et al., 1985). The BB was counted as zero with the first band past the birth band (PBB) as band 1 (0.5 band pairs). Band counts for post OTC (POTC) growth began at the distal

TABLE 2 Linear regression analysis of raw reader POTC band pair counts versus known *Alopias vulpinus* time at liberty.

Best-fit values	Reader 1	Reader 2	Reader 3
Slope	0.8929	0.9381	0.8392
Y-intercept	−0.01603	−0.1484	0.06314
1/slope	1.12	1.066	1.192
95% confidence intervals			
Slope	0.4301 to 1.356	0.5845 to 1.292	0.5405 to 1.138
Y-intercept	−1.102 to 1.070	−0.9782 to 0.6814	−0.6378 to 0.7641
Goodness of Fit			
R^2	0.5955	0.7358	0.7575
Is slope significantly non-zero?			
F	17.67	33.42	37.48
DFn, DFd	1, 12	1, 12	1, 12
p-value	0.0012	<0.0001	<0.0001
Equation	$Y = 0.8929 \times X - 0.01603$	$Y = 0.9381 \times X - 0.1484$	$Y = 0.8392 \times X + 0.06314$

Abbreviations: DFn, degrees of freedom in the numerator; DFd, degrees of freedom in the denominator; POTC, post oxytetracycline.

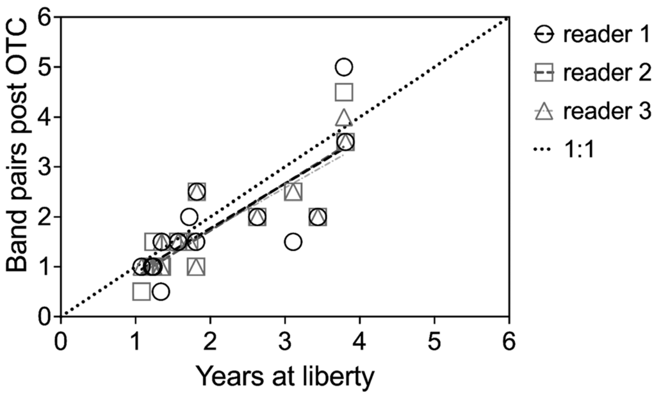

FIGURE 5 Number of vertebral band pairs after the oxytetracycline mark compared to days at liberty for *Alopias vulpinus* at liberty ≥1 year, tagged and recaptured in the northeastern Pacific Ocean (1998–2013) $n = 14$. The dashed lines represent the relationship of band pairs to days at liberty; the dotted line represents a 1:1 deposition rate of one band pair per year.

boundary of the band, which held the OTC mark, such that the band containing the OTC mark was not included in the count (Figure 3). All counts include the marginal band, whether the marginal band appeared translucent or opaque. Together, a translucent band and an opaque band were assigned a count of one band pair, whereas a single band, whether partial or complete, represented a count of 0.5 band pairs. PBB band pairs were always ordered translucent-opaque, as the band distally adjacent to the BB was always translucent. Similarly, when the OTC mark was incorporated into an opaque band, the first band distally adjacent to the OTC-mark was translucent. When the OTC mark was incorporated into a translucent band, POTC band pairs were ordered opaque-translucent.

Multiple images of each sample were provided to the readers, who counted them independently. All sections were read blind without knowledge of shark sample number, size, sex, or time at liberty. Where readers disagreed on band counts after the initial counting round, those samples were assigned a new anonymizing identifier, placed in a random order, and the readers counted a second time.

To compare counts between readers, we used a least-squares linear regression analysis. An *F*-test was used to determine if the slope of the linear relationship between POTC band pairs and known time at liberty was significantly different among the three readers. We tested for age bias in readings after the OTC mark and after the BB using age-bias plots and χ^2 tests of symmetry using the contingency table methods of Bowker (1948) and Hoenig et al. (1995), where a high *p*-value ($p \geq 0.05$) may indicate systemic age bias. Average percentage error (APE) (Beamish & Fournier, 1981) and average coefficient of variation (ACV) (Campana et al., 1995; Chang, 1982) were used to evaluate differences in reader counts using FISHMETHODS (Evans & Hoenig, 1998) and FSH (Ogle et al., 2021) packages in R, version 4.1.0 (R Development Core Team, 2013). The lower the APE and ACV values, the greater accuracy and lower variability of predicted ages.

3 | RESULTS

3.1 | Tagged and recaptured OTC-marked *A. vulpinus*

For the 14 *A. vulpinus* included in this study, average time at liberty was 2.14 years (±0.97 years SD), ranging from 1.08 to 3.81 years. All were juveniles (Natanson et al., 2016; Natanson & Gervelis, 2013) with an average 101 cm LF (±13.42 cm SD) at tagging. Study animals for whom a recapture length was provided ($n = 9$) averaged 137 cm LF (±18.47 cm SD) at recapture (Table 1).

3.2 | Age validation

We found no systematic age bias ($p > 0.05$) in all comparisons for POTC and POBB counts (χ^2-tests of symmetry; Figure 4). Variability among reader counts across all three readers was lower after the BB with an APE of 7.32%, and an ACV of 9.51%, compared to an APE of 10.70% and an ACV of 14.00% for POTC counts. Among readers, all final band pair counts after the OTC were within one band pair of each other, and 93% (13 of 14) of the counts after the BB were within one band pair (Table 1). The slopes of the linear regression analysis conducted for each reader ranged from 0.84 to 0.94 (95% confidence intervals ranging from 0.43 to 1.36, Table 2; Figure 5), and were not significantly different from each other based on an *F*-test ($F = 0.3275$, $p = 0.8054$). The goodness of fit of the linear relationships ranged from 0.60 to 0.76 (Table 2; Figure 5), which indicates that the model explains 60%–76% of the variability in the data around its mean, respectively.

4 | CONCLUSIONS

The band pair deposition rate found in this study contributes information necessary to understand and manage natural and anthropogenic events impacting *A. vulpinus* in the California Current Large Marine Ecosystem. Although we suggest an estimated deposition rate of approximately one band pair per year, the high variation in band pair deposition rates between samples, coupled with regression slopes that are close to the one band pair per year relationship, underscores the need for further investigation to determine band pair deposition rate for the NEPO population with greater certainty.

The average band pair deposition found in the current study is consistent with Natanson et al. (2016) for *A. vulpinus* in the Western North Atlantic (WNA) of the same size range and up to approximately 14 years of age (validated through bomb radiocarbon dating). However, this band pair deposition rate should be applied only to *A. vulpinus* less than or equal to the size range examined in this study (*c.* 170 cm LF). Using bomb radiocarbon dating (Hamady et al., 2014), Natanson et al. (2016) found the band pair deposition rate may change over their lifespan in the WNA; they found a deposition rate of one band pair per year up to approximately age 14, with a subsequent decrease in deposition rate. This observation led to an update to previously published growth curves for the WNA population, and a realization that longevity had been underestimated using band pair counts. A comparable study could examine if band pair deposition rate varies over time within *A. vulpinus* in the NEPO.

Challenges associated with OTC tag and recapture studies include low rates of recapture, poor or incomplete data upon recapture, and uncertainty of time required for OTC to incorporate into calcifying tissues. This study would have benefited from an increased sample size from larger *A. vulpinus* and more samples with longer times at liberty. The greatest time at liberty for recaptured sharks in this study was 3.8 years, and the largest shark was 168 cm LF at the time of recapture, whereas fishery observer data in the NEPO include animals up to 283 cm LF (Data S1, Supplementary Figure S2). With more than 1500 juvenile *A. vulpinus* OTC-tagged during NOAA surveys through 2017 and longevity estimates of 25 years or more (Smith et al., 2008), additional recaptures may occur. However, time and area closures implemented for the protection of reproductively mature female *A. vulpinus*, and for the protection of other species, combined with reduced fishing pressure in the California-based drift gillnet fishery, now make the capture of large *A. vulpinus* less common.

A. vulpinus are often reported recaptured at fish camps on beaches in Mexico, or by fishers at sea who are untrained in recapture data collection. Lengths measured in these situations can be from sharks in any of a variety of processing conditions, making recapture lengths unreliable, and recapture locations inaccurate or missing. Increased reporting and accuracy of recapture lengths and locations would have increased our confidence in the return information; however, because we were not modeling growth, the missing information did not negatively impact this study.

Though time required for OTC incorporation in thresher sharks is unknown, previous studies have estimated between 6 and 72 days for other shark species (Branstetter, 1987; Natanson et al., 2002 and Tanaka, 1990). Large variation in uptake rates may be due to variability in injection doses, differing tissue injection sites, and uncertain success in OTC entering the tissue. In the present study, returned vertebrae of *A. vulpinus* at liberty for 1 through 20 days showed fluorescence on the outer margin, though these marks did not appear as a cohesive, discernable ring. It is unclear how much time is required for a cohesive discernable mark to form after OTC injection, if incorporation time is consistent across individuals, and if errors in OTC injection doses impact OTC-mark formation. When injecting *A. vulpinus* in this study, OTC was observed dripping out of the injection site in varying amounts, which indicates potential variation in exact dose delivered to the target tissue. A vertebra from an *A. vulpinus* at liberty for 80 days exhibited a fluorescing OTC mark distinct from the marginal tissue. For age validation in this study, we used samples from sharks at liberty for 1 year or more, ensuring at least one full annual band deposition cycle. We also examined a total of 37 OTC-marked vertebrae from animals at liberty for 0.5 years or more and obtained similar results (unpubl. data).

The relationship between band pairs deposited and time at liberty ranged from 0.84 to 0.94 (95% confidence intervals ranging from 0.43 to 1.36; Table 2; Figure 5) among the three counters. However, we found many *A. vulpinus* included in our study have fewer or more bands than would be predicted by a one band pair per year deposition rate. This may, at least in part, be due to variation among reader counts (Figure 4; Table 2), or counting methodology in general. Although no systemic bias was found among readers, reader count discrepancies as little as 0.5 to 1 band pair introduce uncertainty due to the relatively short overall life of the study animals. There is greater relative uncertainty associated with a one band pair discrepancy in a study of juvenile sharks than for the same discrepancy in a study of mature sharks with a greater number of total band pairs. Given that a band's completeness is signaled by the initiation of the next band, inclusion of the marginal band in band counts could inflate deposition rate calculations, particularly for short times-at-liberty and young animals. Conversely, excluding the band that includes the OTC mark may bias the time-at-liberty band count toward under-counting deposition rates. We included the marginal band in PBB and POTC counts and did not include the band containing the OTC mark in POTC counts, potentially introducing some bias into the analysis.

OTC tag and recapture is one of few techniques for validating band pair deposition rates in sharks. However, it requires great effort and financial resources to capture, handle, and chemically mark large numbers of individuals, and return rates are frequently low (e.g., *c*. 5% in this study). Additionally, there can be limited or inadequate fluorescence of OTC in injected specimens (e.g., 47% in spiny dogfish vertebrae; James et al., 2021). Although additional study is needed, these preliminary findings of band pair deposition for juvenile *A. vulpinus* in the NEPO can be used as a foundation for further research, and inform biologists, modelers, and fishery managers as they develop and refine growth estimates used in stock assessments and provide informed management advice.

4.1 | Future directions

The age validation information found in this study provides a foundation for juvenile *A. vulpinus* age and growth studies moving forward, and can be applied to vertebrae of size-relevant individuals to improve growth curves. Further studies on maturity of *A. vulpinus* in the NEPO are needed to help resolve uncertainties, explain potential differences between regions, and to determine variation between individual *A. vulpinus* on a larger scale. Future age validation studies of *A. vulpinus* in the NEPO, particularly for large sharks, would support a more complete understanding of *A. vulpinus* population dynamics in the NEPO.

AUTHOR CONTRIBUTIONS

L.N.S., R.J.D.W., and S.K. conceptualized the study and the methodological approach, building on existing NOAA monitoring and survey protocol for *A. vulpinus*. L.N.S. conducted the laboratory investigation under supervision from R.J.D.W. Fieldwork was conducted by S.K., L.N.S., R.J.D.W., and S.W.F.S.C. staff. Data were analysed by L.N.S., S.K., and J.A.M. The original draft was written by L.N.S. with significant contributions by R.J.D.W., S.K., and J.A.M. Funding was in part provided by the National Oceanic and Atmospheric Administration Cooperative Research Program.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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