NIWA

Taihoro Nukurangi

Climate, Freshwater & Ocean Science

Age estimation of white sharks (*Carcharodon carcharias*)

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Project objective

Provide age and growth estimates for New Zealand white sharks

White shark age and growth

- Not assessed for NZ sharks; life history parameters poorly defined due to relative rarity and difficulties associated with sampling large sharks

- Growth rates vary regionally:
  - males reach maturity between 3.1–4.1 m total length (TL)
  - females reach maturity between 4.0–5.0 m TL

- Initial age estimates from Northwest Atlantic, Northeast Pacific, and Western Indian Oceans suggested white sharks reached maturity 7–15 years and maximum longevity of 18–30 years

- Recent validated age estimates using bomb radiocarbon dating indicated white sharks have slow growth rates and are long lived: female maturity 30–33 years, maximum age up to 73 years (validated to ~40 years)

- Some unvalidated Australian data suggests similar rates of growth and longevity to initial estimates

White shark age and growth

- Knowledge of age and growth parameters is vital to estimate population growth rates and other important demographic parameters such as maximum age, age-at-maturity, and productivity

- Age and growth parameters are also essential inputs for developing technologies (e.g., genetic close kin mark-recapture analyses, DNA methylation ageing)

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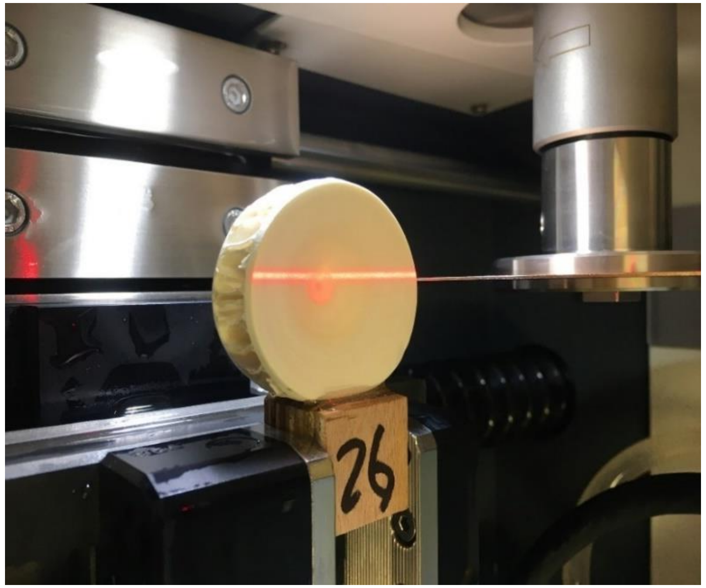
Methods: Sample collection

- Vertebrae were obtained from white sharks reported dead from sources including commercial and recreational fishing vessels (bottom setnet, longline, cray pot, bottom trawl, rod and line), and beach cast specimens from 1991 to 2021

- Previously processed microscopy slides were available for three individuals collected in 1991 and 1996. These slides were in poor condition, but two were included here because they included some of the largest available sharks (4.8, 5.3 m TL)

Methods: Sample prep

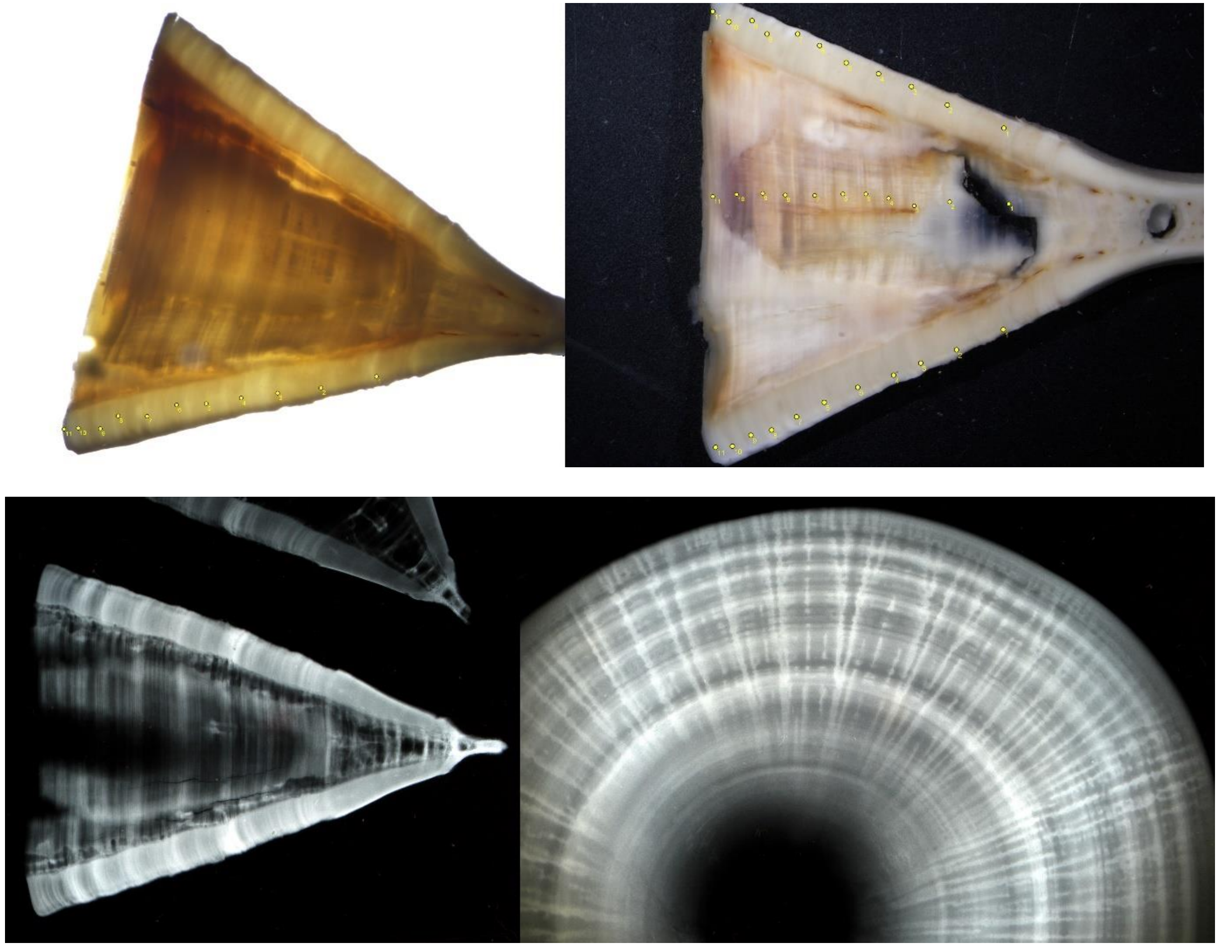
- Same preparation technique used in previous age and growth studies NZ sharks (removal excess muscle and connective tissue, briefly bleached, dried, sectioned)
- Vertebral radius (VR) measurements were taken from the largest visible vertebrae (post-defrosting, in mm). The TL-VR relationship is used to assess the allometric relationship between vertebral and body growth; estimate length for sharks with no length measurement
- Vertebrae were sectioned in the frontal plane (medio-laterally), producing 'bowtie' thin sections (ranging 0.6–0.9 mm thick)



Methods: Sample prep

- Each thin section was photographed at a variety of magnifications using reflected and transmitted light

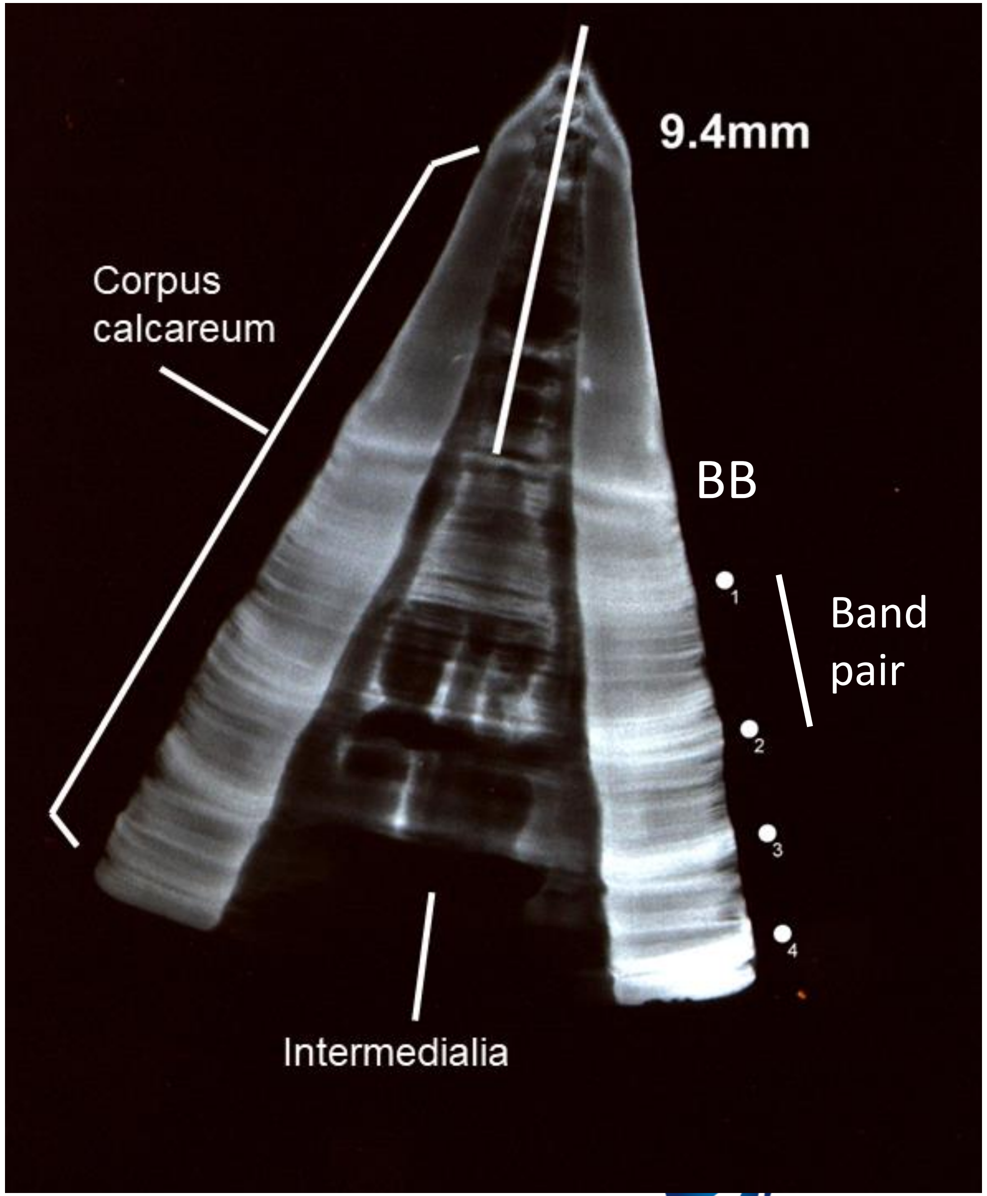
- All thin sections and half centra were x-rayed and digitally captured



Example of thin section images under transmitted light (top left panel) and reflected light (top right panel), and an example of digitally captured images from x-rays for both thin section (bottom left panel) and the correlate whole centrum face (bottom right panel)

Methods: Age estimates

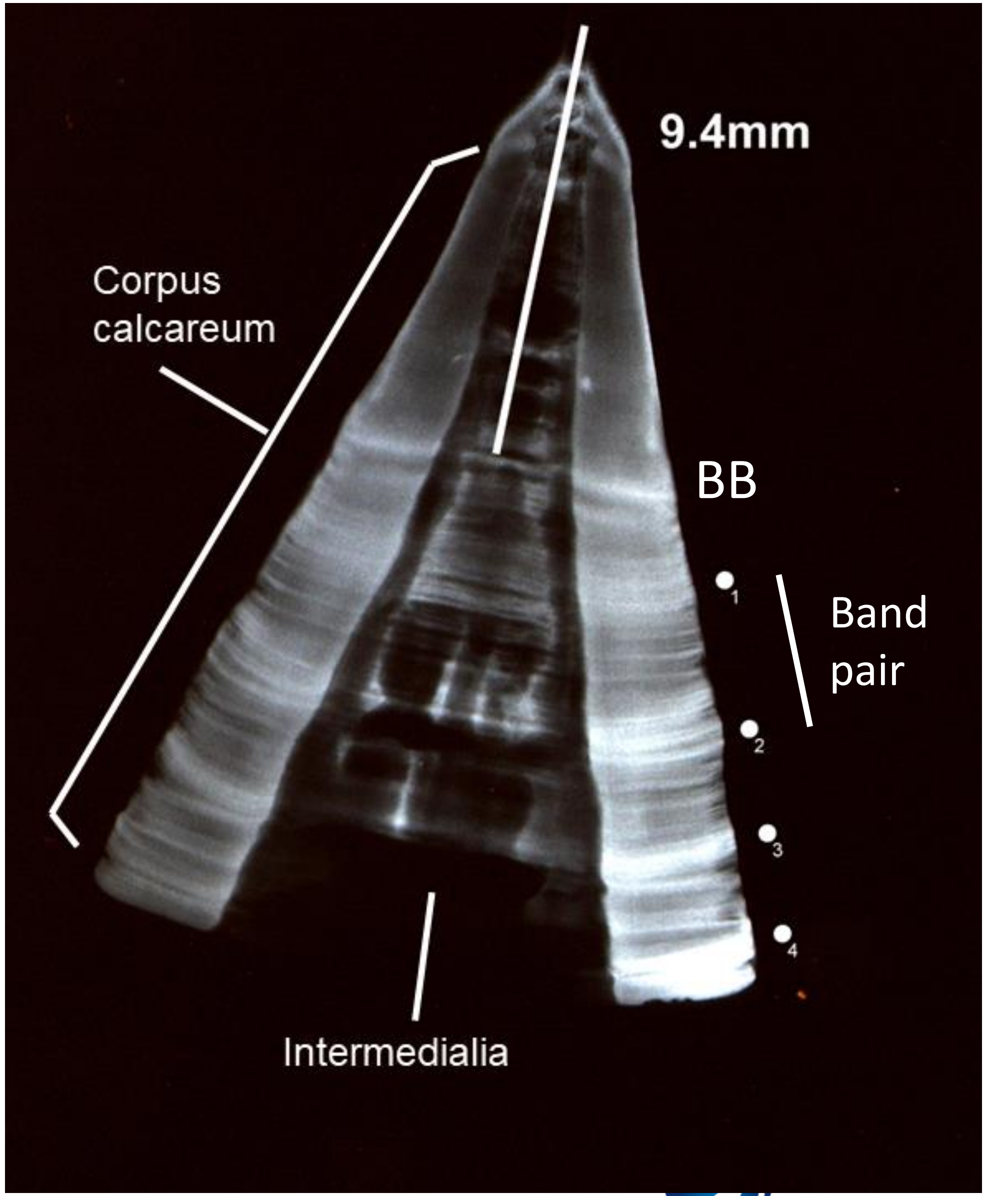
- Reference ages were not available for this study
- Identify birth band (BB) - prominent contrasting band in the centrum deposited about or soon after birth; important to determine where counts should begin
- BB estimated to occur at ~9.4 mm from the focus, used as a guide here if BB location uncertain (BB occurred at 9.2-9.8 mm)
- Growth bands were defined as a band pair consisting of one translucent and one opaque band; assumed to signify one year of growth
- Band pairs near the margin became much narrower and difficult to resolve in large sharks; can lead to underestimation of age
- Distinct bands on the corpus calcareum, while bands within the intermedialia were usually less distinct



Methods: Age estimates

- Two readers carried out counts, both experienced in reading shark vertebrae
- Reader 2 also counted bands from the whole vertebra (half centra)

- Between-reader age-estimation bias and precision were explored with a frequency distribution of the age differences, an age-bias plot, and plots of the average percent error (APE) and the mean coefficient of variation (CV)



Methods: Analyses

- Growth curves were fitted to the length-at-age data using the R package *FSA* which fits non-linear curves using the R package *nlstools*
- Data were fitted with the von Bertalanffy growth function (VBGF) to make direct comparisons with other white shark growth analysis from the Pacific and Indian Oceans

- Marginal increment ratios (MIRs) can be used as a validation technique to determine the periodicity of band formation
- MIRs are calculated by comparing the widths of the ultimate and penultimate band pairs, mean values plotted by month or season of capture

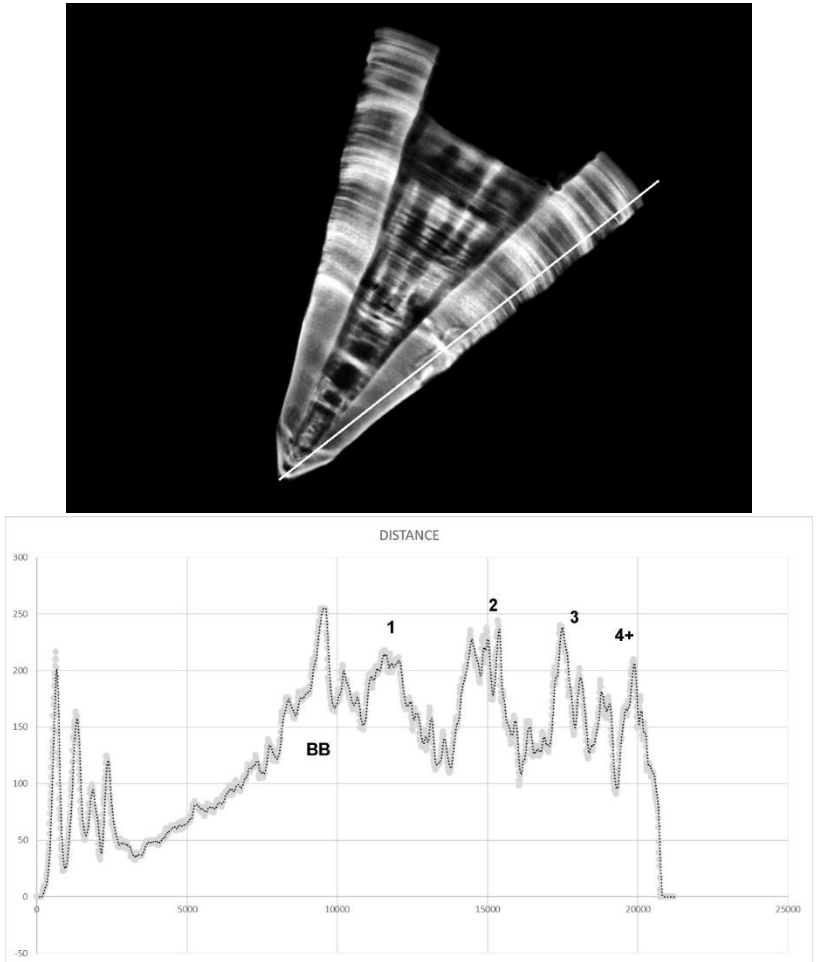
- A preliminary MIR analysis was conducted here and included all samples with fully formed band pairs, excluding slide samples for the poor readability
- Mean ratio values were not calculated due to small sample sizes

Methods: micro-CT and density profiles

- Three whole vertebrae (one small, medium, and large) were imaged with micro-computed tomography (micro-CT) equipment (University of Auckland, Flinders University)

- To further assist in the interpretation of band formation, line profiles were created down adjacent arms of the corpus calcareum in transverse view to produce a graph showing peaks where grey level intensity of the image signal (i.e., a proxy for ring or band density)

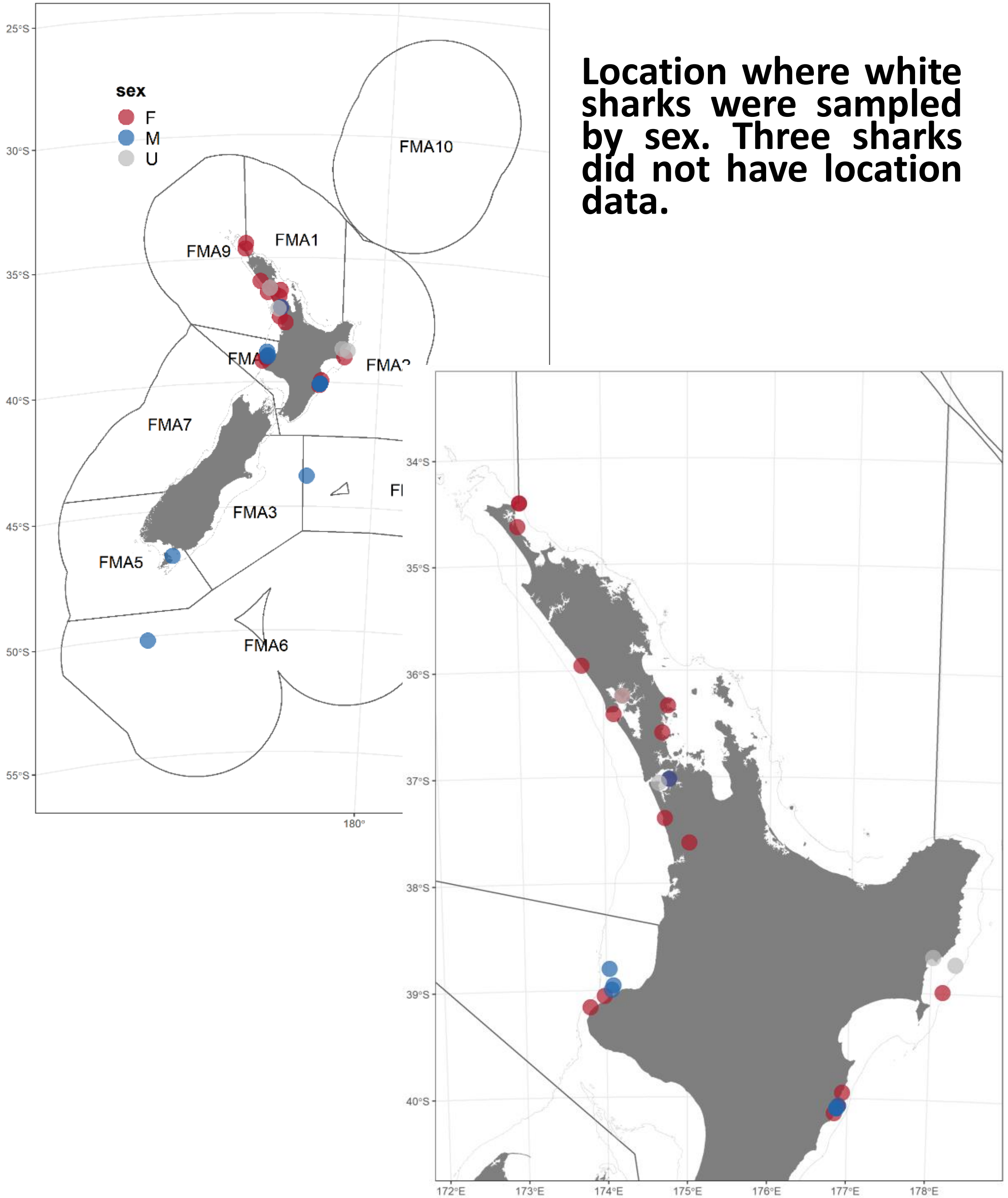
- Peaks observed in the graph were inferred to corelate to band patterns



Thin section x-ray of ururoa_2 and its density profile (taken along the white line) indicating the number of band patterns. BB = birth band.

Results: Geographic scope

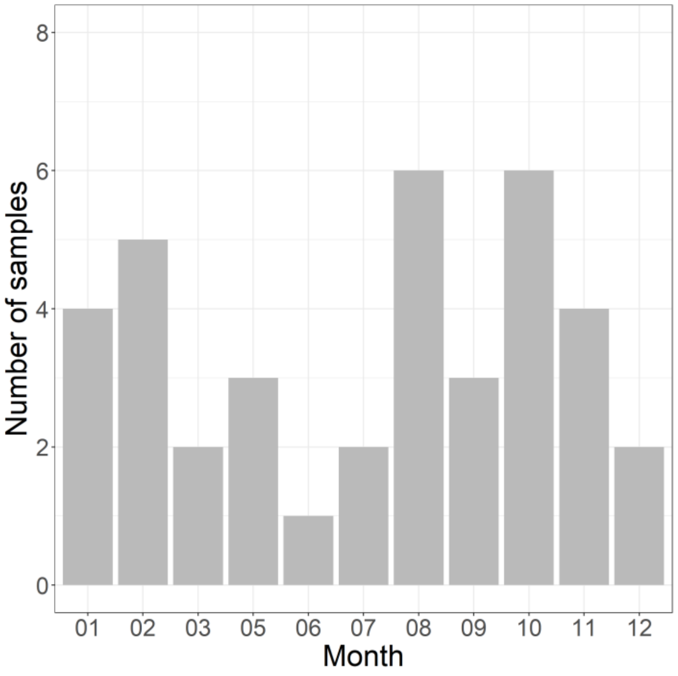
- 38 individual white sharks
- Most white shark samples were collected around the North Island
- Samples were taken in 21 of the 30 years (1991-2021), with no more than four samples reported from a single year (2005)

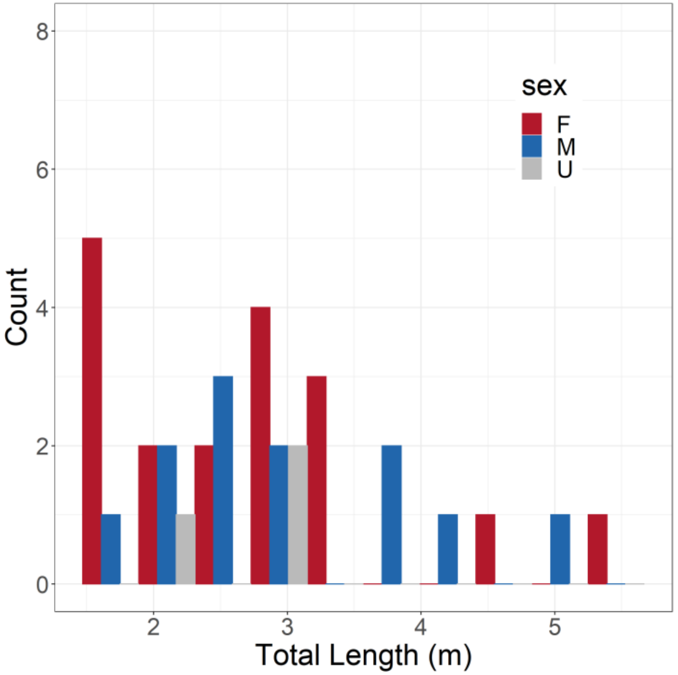


Location where white sharks were sampled by sex. Three sharks did not have location data.

Results: Sample composition

- Sharks were sampled throughout the year, in every month except April; mostly in spring (Sept-Nov, 13 samples) and summer (Dec-Feb, 11 samples)
- 20 female sharks ranging in lengths from 1.52 to 5.36 m TL (mean = 2.77 m TL)
- 12 male sharks ranging in lengths from 1.87 to 4.85 m TL (mean = 2.68 m TL)
- Six unsexed sharks ranging in lengths from 2.26 to 3.0 m TL (mean = 2.84 m TL)
- Maturity estimates only available for seven sharks (one mature female (5.36 m TL) and six immature sharks)



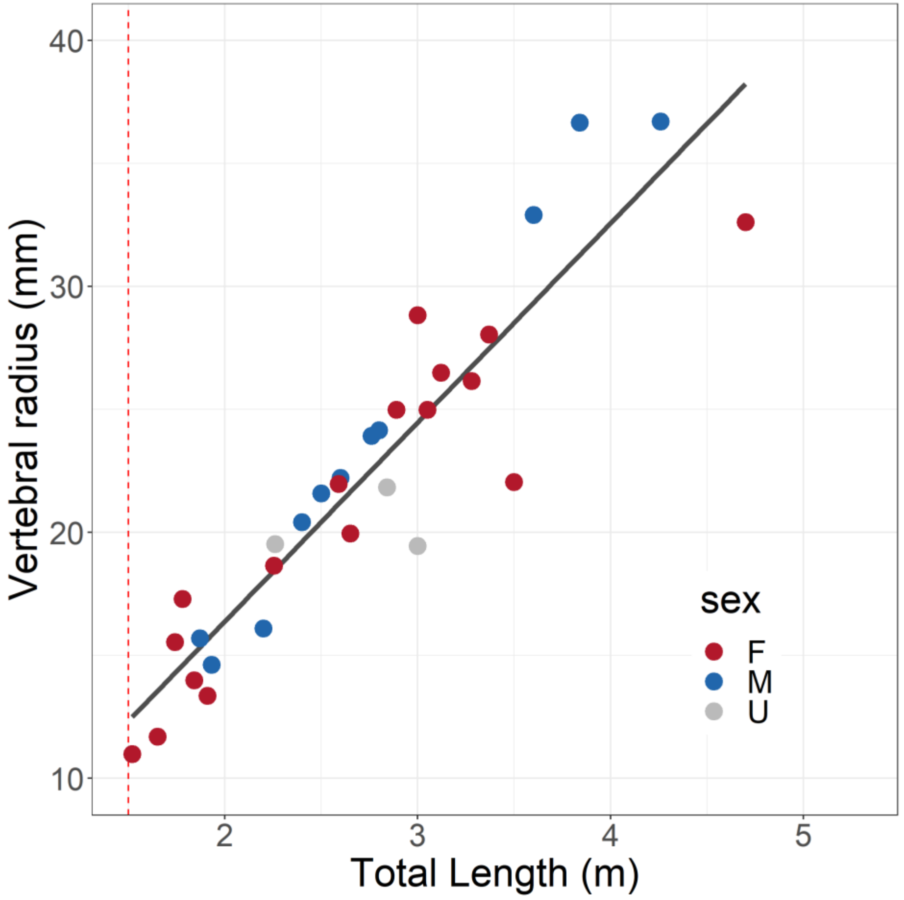


Results: TL-VR relationship

- The relationship between total length and vertebral radius was significant for males ($R^2 = 0.976$, $p < 0.001$) and females ($R^2 = 0.847$, $p < 0.001$), but there was no significant difference between the regressions ($F_{1,25} = 3.291$, $p = 0.081$)
- Thus, the data was combined to give the following regression based on 31 samples (11 males, 17 females, 3 unknown sex):

$$TL = 8.093VR + 0.191 \ (R^2 = 0.85)$$

- This regression was used to estimate the length for two female sharks and three unsexed sharks (estimate lengths of 1.8 m to 4.6 m)



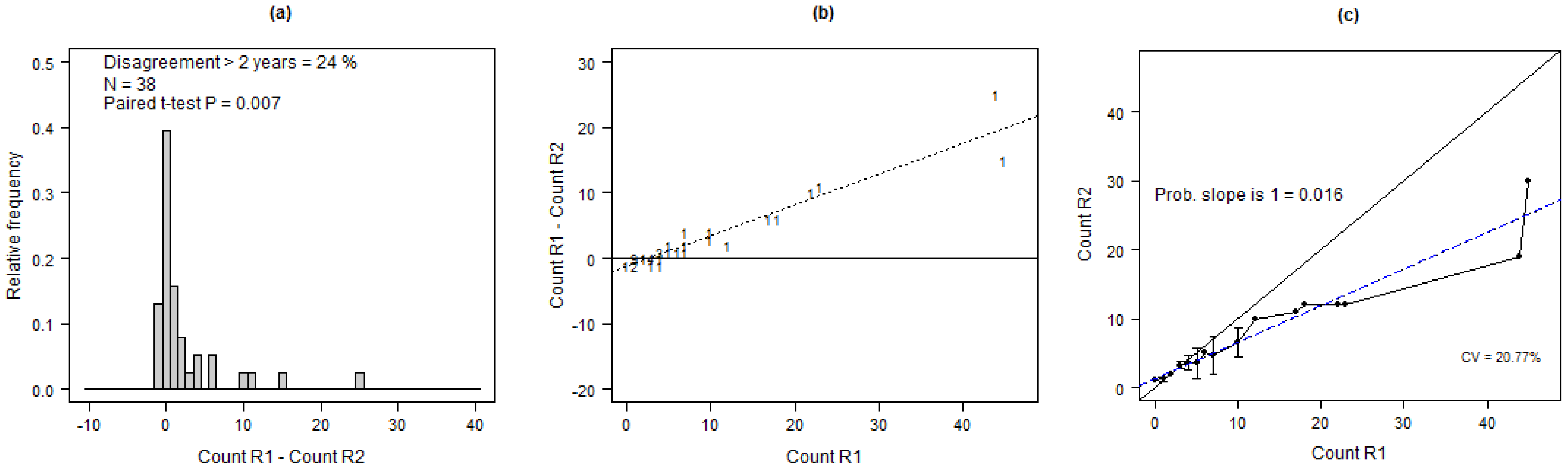
Results: Vertebrae reading

- Vertebrae were difficult to read, particularly for old sharks
- Additional fine growth rings were present in most vertebrae and the distinction between these rings and bands was not always obvious; increments may be associated with finer increments of time, such as lunar or monthly pattern
- Readings were highly variable between readers, with an overall CV of 20.77% and APE of 14.69%, and absolute differences (Reader 1 minus Reader 2) ranging from –1 years to 25 years (mean 2.39)

Thin section for ururoa_45 with annotated counts by Reader 1 (bottom panel)

Results: Vertebrae reading

- Readings of 76% of the vertebrae agreed within ±2 years
- Reader 1 showed a tendency to count more bands than Reader 2, but this was non-significant (paired t-test, $p > 0.05$) (Figure AB)
- Age bias occurred in larger sharks, and the slope of the age-bias regression was significantly different from 1 (slope = 0.532, $p = 0.016$; Figure C)



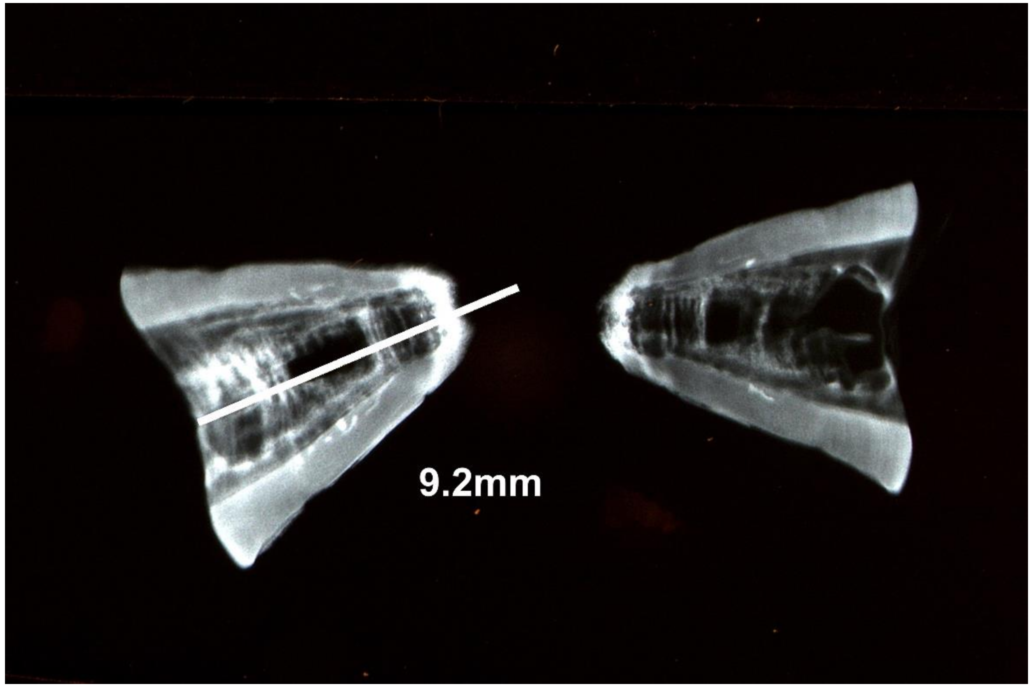
Analysis of vertebral band count differences between Reader 1 and Reader 2. Dotted and dashed lines are fitted linear regressions. CV, coefficient of variation; APE, average percentage error.

Results : Vertebrae reading

- There was no agreement (±2 years) between readers for sharks greater than 10 years of age (n = 6)
- Agreement (±2 years) for age estimates was improved to 91% when only considering sharks up to 10 years of age
- Some improvement with the overall CV (17.51%) and APE (12.38%), and absolute differences (Reader 1 minus Reader 2) ranging from –1 years to 4 years (mean 0.562)

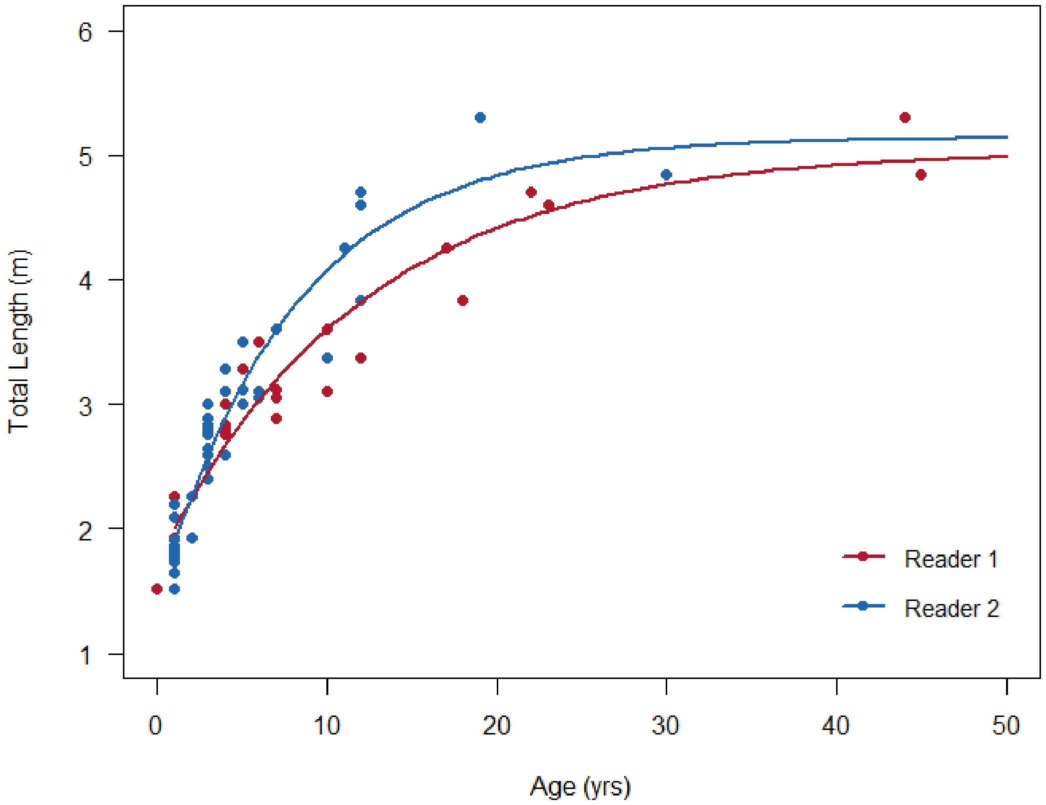
Results : Vertebrae reading

- Most (74%, n = 28) sharks were estimated to be under the age of 5 and a quarter (n = 10) of sampled sharks were estimated to be 1 year of age
- One shark (1.53 m TL) had no fully formed growth bands or distinct birth band, and was likely a neonate
- Maximum age estimates from the band counts for Reader 1 and Reader 2, respectively, were 30 and 45 years for males (4.85 m TL) and 19 and 44 years for females (5.36 m TL)



Results: Growth Curves

- VGBF growth curves were fitted to the combined-sex age estimates for Reader 1 and Reader 2 separately
- Assuming annual deposition of band pairs, white sharks showed fast growth during their first 2-3 years
- For age estimates for both readers, growth appears to be almost linear in the first few years of age
- For Reader 1, white sharks were estimated to reach 2.8 m by five years, and 3.6 m by 10 years
- For Reader 2, white sharks were estimated reached 3.2 m by 5 years, and 4.08 m by 10 years
- In either case, sharks nearly doubled their birth length of ~150 cm within five years; growth slowed by years 5–6

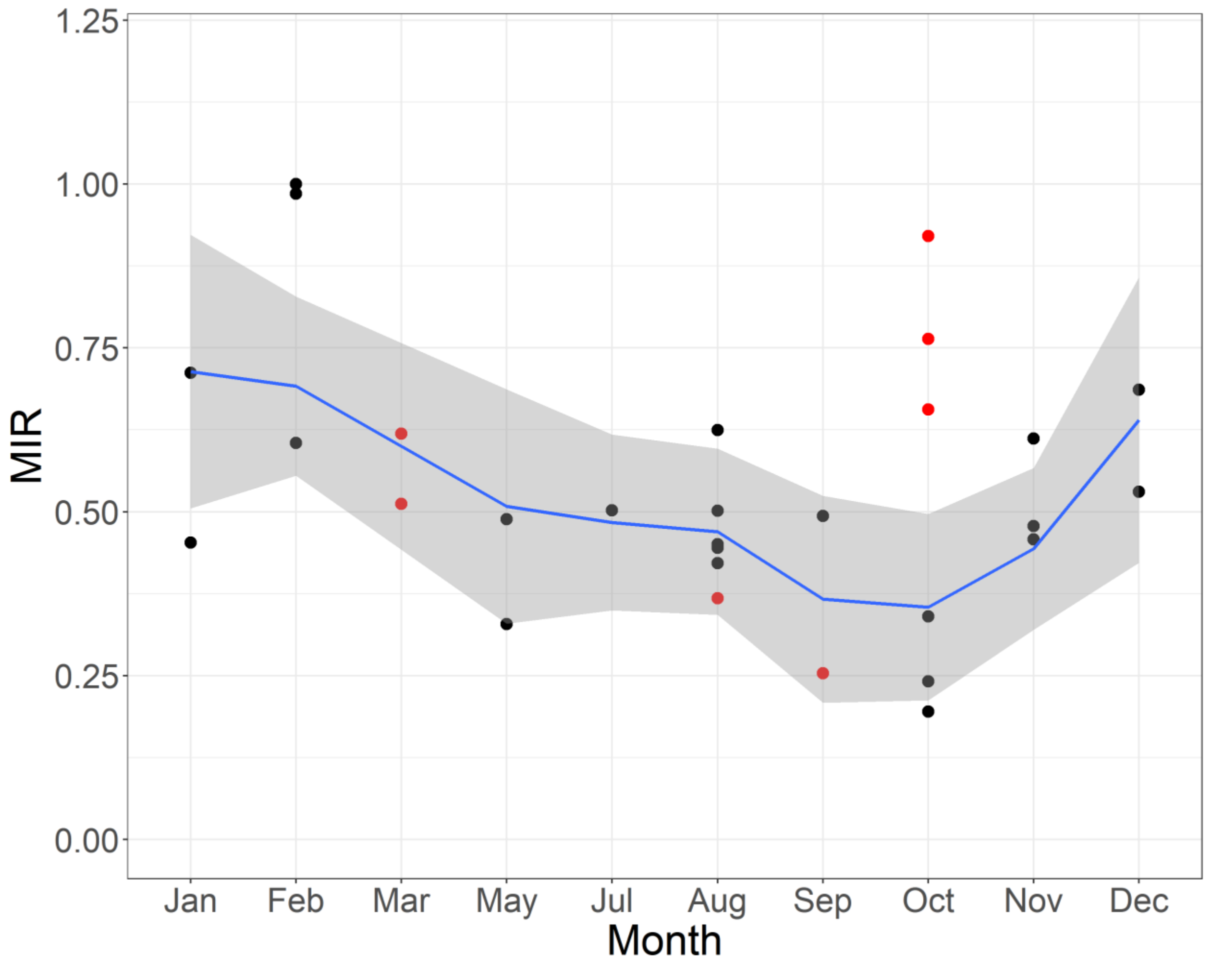


White shark growth parameter estimates

Region	n	Sex	L_∞ (m, TL)	k (year^{-1})	t_0 (years)	L_0 (m, TL)
New Zealand (Reader 1)	38	Combined	5.035 (4.461–6.897)	0.083 (0.066, 0.184)	-5.05 (-4.78, -1.70)	1.734
New Zealand (Reader 2)	38	Combined	5.141 (4.414–6.847)	0.125 (0.060, 0.183)	-2.63 (-4.50, -1.63)	1.441

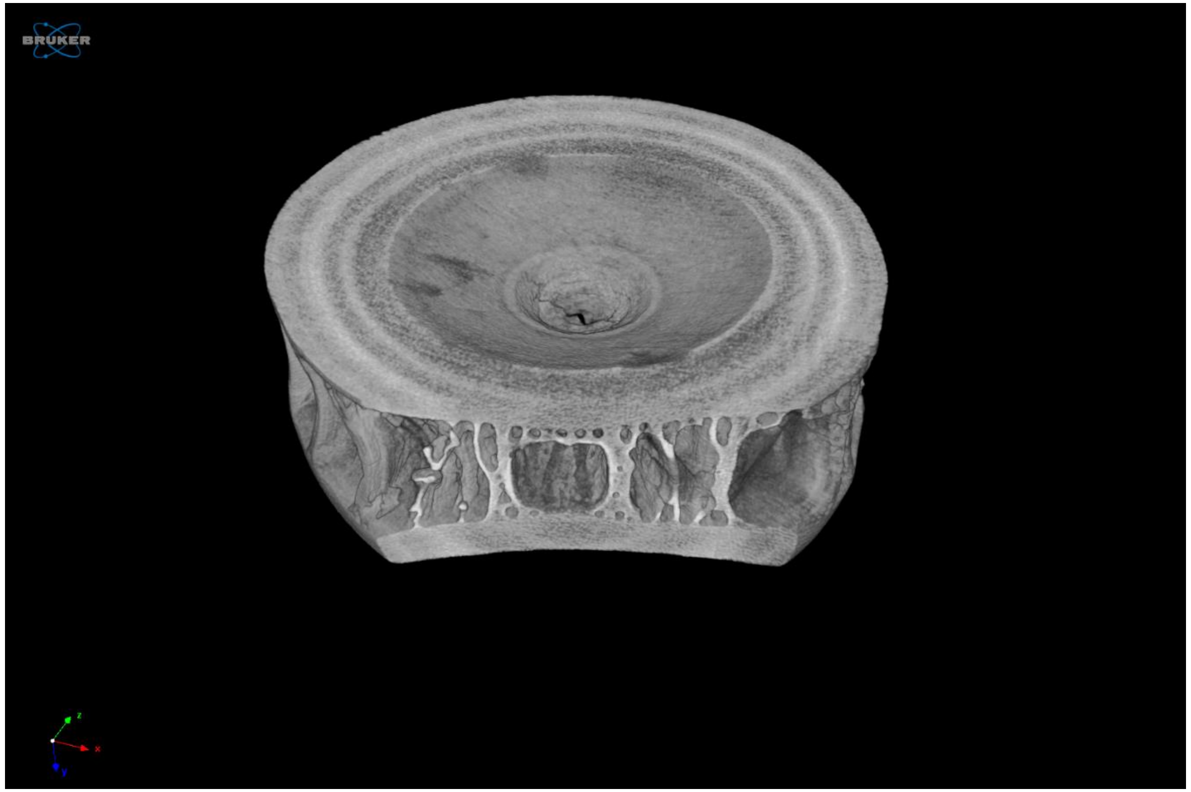
Results: MIR

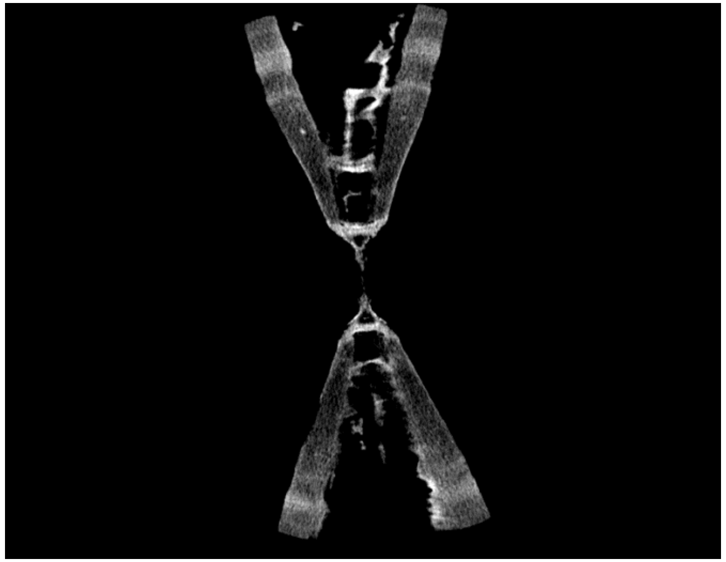
- Marginal increment ratios were calculated for 30 sharks
- MIR was highest in summer months (December to February), declined in growth formation leading into the autumn and winter months, and increased leading into spring (October onwards)
- October showed the biggest spread in ratio measurements; divided into young sharks (3–4 years old) with lower ratios and older sharks (6+ years) with higher ratios

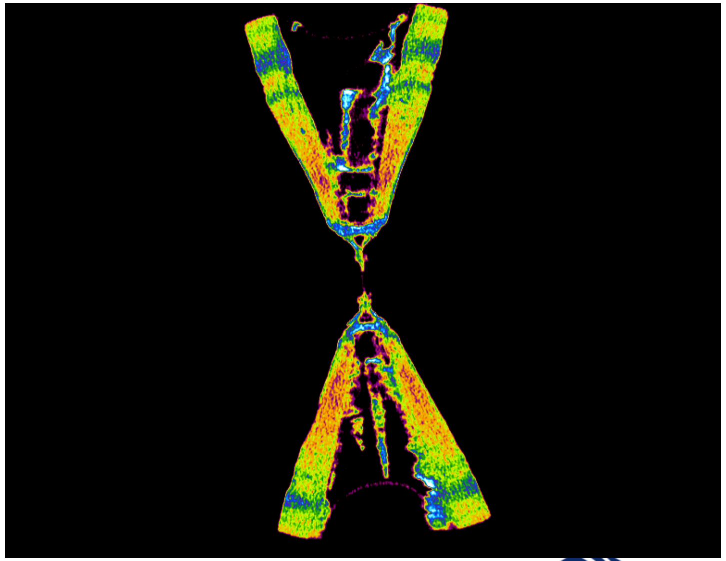


Marginal increment ratio (MIR) by month of capture. Black dots indicate sharks aged 5 years or less and red dots indicate sharks aged older than 5 years. Data for sharks aged 5 or less were fitted with a moving regression (LOESS)

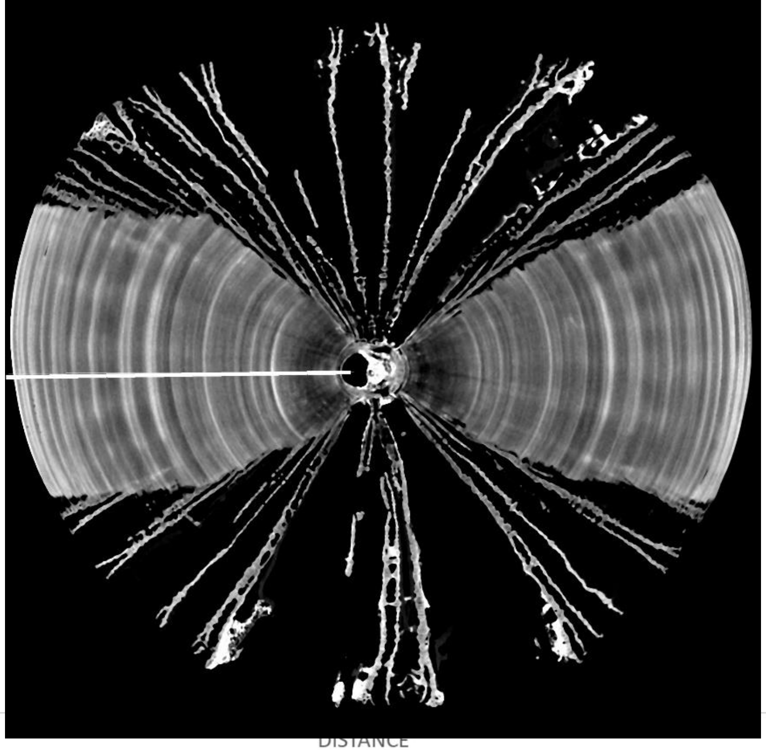
Results: micro-CT



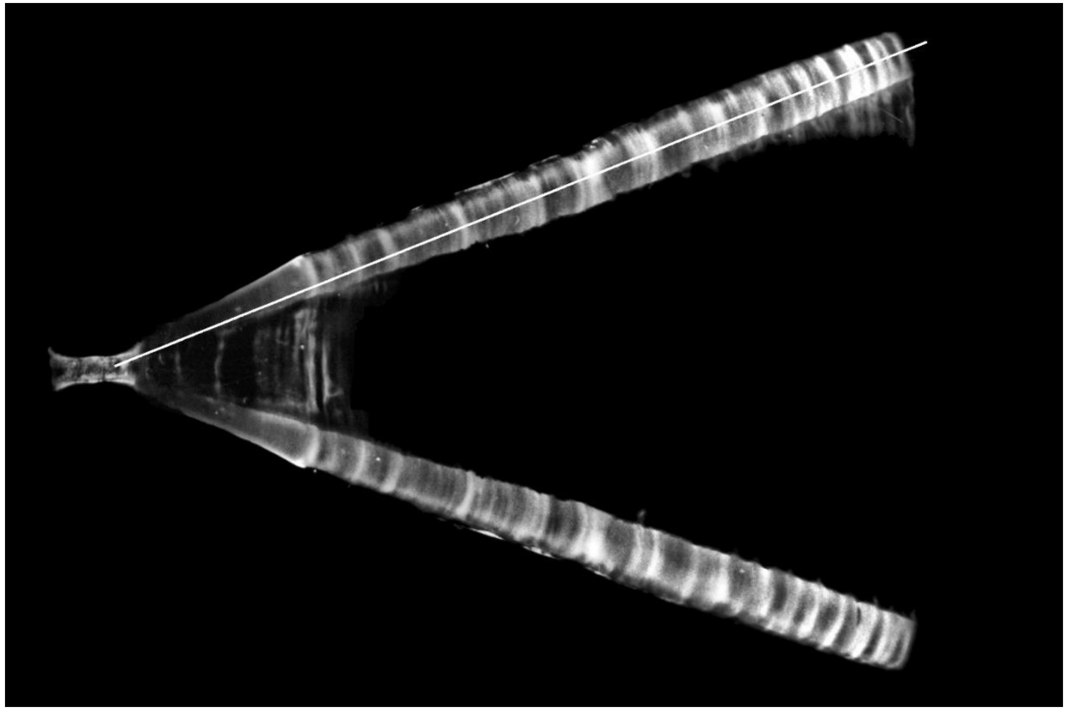


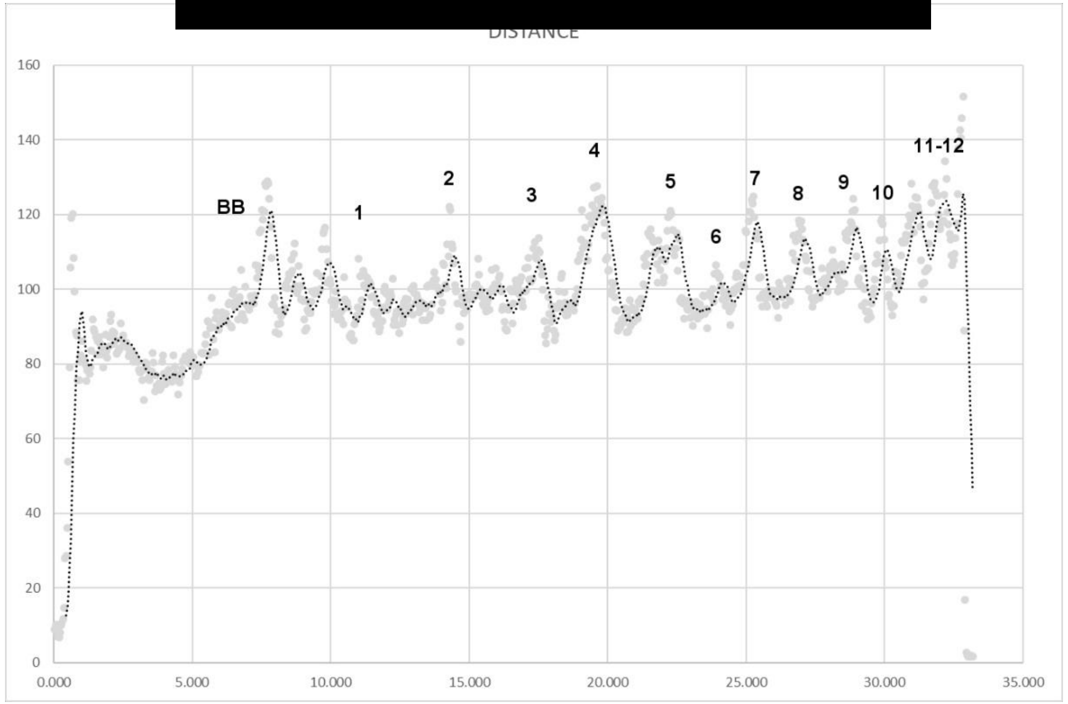


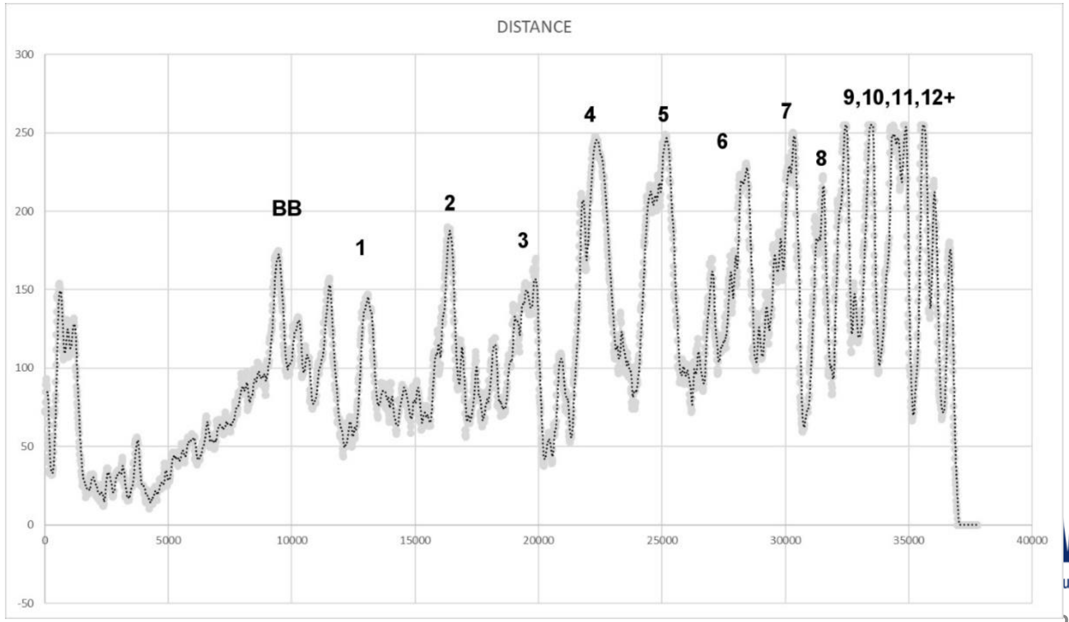
Micro-CT



X-ray







Summary

- Age and growth were estimated for NZ sharks for the first time using traditional ageing methods (vertebrae band pair counts) and micro-CT scans
- Samples (n=38) were collected over a period of 30 years
- Nearly half of the individuals were young (1–2 years old), only six sharks were estimated >10 years of age
- Vertebrae were not easy to age visually, particularly for older individuals
- There was strong agreement between readers for age estimates of young sharks, but large disagreement for older sharks
- New Zealand white sharks appear to be relatively fast growing initially (double birth size within 5 years), and are possibly long-lived (unvalidated age estimates of >40 years)

Comparison with other studies

Table 1. White shark growth parameter estimates from studies conducted in the Pacific and Indian Oceans. Standard errors for estimates from this study are provided in brackets.

Region	*n*	Sex	L_∞ (m, TL)	*k* (year^{-1})	t_0 (years)	L_0 (m, TL)	Reference
New Zealand (Reader 1)	38	Combined	5.035 (4.461–6.897)	0.083 (0.066, 0.184)	-5.05 (-4.78, -1.70)	1.734	This study
New Zealand (Reader 2)	38	Combined	5.141 (4.414–6.847)	0.125 (0.060, 0.183)	-2.63 (-4.50, -1.63)	1.441	This study
Southern Australia	51	Combined	732.3 (high) 659.8 (low)	0.042 (high) 0.071 (low)	4.173 (high) -2.330 (low)	NA	Malcolm et al. (2001)
Southern Australia	79	Combined	7.466	0.053	-3.80	1.400	O'Connor (2011)
East Pacific Ocean (California)	21	Combined	7.637	0.058	-3.53	1.250	Cailliet et al. (1985)
Northwest Pacific Ocean (Japan)	21	Male and Female	4.550 (M), 6.067 (F)	0.196 (M), 0.159 (F)	-1.92/-1.63	1.425 (M) 1.507 (F)	Tanaka et al. (2011)
Southwest Indian Ocean (South Africa)	112	Combined	6.860	0.065	-4.40	NA	Wintner & Cliff (1999)

Future work

- Continued collection of biological sampling, particularly of larger sharks
- Investigate alterative non-lethal means of ageing

  Emerging technologies, such as DNA methylation, have been successful in estimating fish age via tissue samples but validated ages are required

- Complete a New Zealand-Australia white shark growth study

  Current interest in revisiting and improving previous Australian analyses; possibility of validating ages

- Assessment of vertebrae elemental composition

  Vertebral chemistry can be a used as natural tag to reconstruct movement and environmental histories of sharks (e.g. temporal and spatial movements patterns from coastal to oceanic habitats); may provide insight into movement patterns, particularly for juvenile individuals, for which there is currently few data available

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