



Age and growth determination of three sympatric wobbegong sharks: How reliable is growth band periodicity in Orectolobidae?



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ABSTRACT

The determination of age for large, harvested species such as chondrichthyans is important to the estimation of growth and other key life history parameters such as natural mortality, age-at-maturity, longevity, and recruitment. Vertebrae from 760 wobbegongs (275 *Orectolobus ornatus*, 232 *O. maculatus*, and 253 *O. halei*) were collected between June 2003 and December 2007 at seven locations in eastern Australia (Queensland and New South Wales) to estimate growth parameters for these species. A multi-model inference (MMI) information theoretical approach including four candidate models, with back-calculated estimates of length in earlier life stages to account for limited numbers of pup and juvenile wobbegongs, was used to determine the most appropriate growth model for each species. The models that combined observed and back-calculated lengths-at-age did not provide a better fit than the model using observed lengths-at-age data only. Taking into account biologically meaningful estimations of L_{∞} and k , the models with the best fit to the data were the logistic growth function for *O. ornatus* and *O. halei*, and the von Bertalanffy growth model for *O. maculatus*. Using these models, growth parameters obtained were: 999, 1630 and 2128 mm total length for L_{∞} and 0.19, 0.09 and 0.20 for k , while the maximum number of growth bands was 20, 22, and 27, for *O. ornatus*, *O. maculatus*, and *O. halei*, respectively. All three species were monomorphic, with similar growth rates for males and females. Verification and validation undertaken using edge and marginal increment analyses, as well as chemical marking of captive and wild wobbegongs, suggested that growth band deposition in orectolobids is more likely to be linked to somatic growth than seasonality. This study is the first to use chemically marked wild Orectolobiformes to investigate growth band deposition rate. Five orectolobid species have now been shown not to deposit growth bands following a synchronous annual pattern, in contrast to that inferred for most other chondrichthyan species. The growth parameters estimated in this study are crucial for stock assessments and for demographic analyses to assess the sustainability of commercial harvests.

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1. Introduction

The determination of age for large, harvested species such as chondrichthyans, is essential to the accurate estimation of growth and other key life history parameters such as natural mortality, age-at-maturity, longevity, and recruitment (Pauly, 1987). Growth rates and age determination are key components of fisheries research (Cailliet and Goldman, 2004), and are required for most fisheries stock assessments based on age-structured population models

(Pauly, 1987). Recent increases in chondrichthyan exploitation around the world (Bonfil, 1994; Field et al., 2009; Lack and Sant, 2006; Stevens et al., 2000) have highlighted the need for life history studies and especially those focusing on age and growth. Crucially, inaccurate age determination can lead to major errors in stock assessment and poor estimation of resilience to fishing pressure, leading to overexploitation (Campana, 2001; Hoenig and Gruber, 1990; Hoff and Musick, 1990; Musick, 1999; Officer et al., 1996).

Wobbegongs (family: Orectolobidae) are demersal sharks, found in temperate and tropical Western Pacific waters (Compagno, 2001; Last and Stevens, 2009). Twelve species of wobbegong shark have been identified worldwide, albeit taxonomic uncertainties remain. Ten of these species are found in Australian coastal waters, with three occurring in New South

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Wales (NSW): *Orectolobus ornatus*, *O. maculatus*, and *O. halei*, and an additional two in Queensland (Qld) *Eucrossorhinus dasypogon* and *O. wardi* (Corrigan et al., 2008; Huveneers, 2006; Last et al., 2010; Last and Stevens, 2009). In NSW, wobbegongs have been commercially targeted within the Ocean Trap and Line Fishery since 1991, to be sold as flake. From 1990/1991 to 2000/2001, the catch reduced from ~150 tonnes to ~65 tonnes per year, representing a decrease of more than 50% in a decade (Pease and Grinberg, 1995; NSW DPI, 2006). Since then, catches remained stable at around 60 tonnes per year until 2008/2009, when the annual catch fell to 20 tonnes per year. This latter reduction of catch resulted from fishing legislation introduced in 2007 that limits the maximum bag number of wobbegongs for commercial fishers to six per day (Rowling et al., 2010). Although the catches seem to have stabilized since the new regulations were implemented, the ability of wobbegongs to withstand fishing pressure remains in question. This uncertainty is exemplified by the recent nomination of the spotted wobbegong (*O. maculatus*) for listing as a threatened species under the *Environment Protection and Biodiversity Conservation Act 1999* (the EPBC Act). An assessment of wobbegong resilience to fishing pressure is necessary to inform management decisions such as the proposed EPBC listing, for which accurate determination of age and growth parameters is required.

Concentric growth bands have been documented in the vertebral centra of most chondrichthyans for nearly a century (Ridewood, 1921), and are often used for ageing. The only study to report growth parameters for Australian wobbegongs provided estimates for *Orectolobus hutchinsi* and *O. halei* (misidentified as *O. ornatus*) collected from waters off Perth, Western Australia (Chidlow, 2003; Chidlow et al., 2007). Chidlow et al. (2007) attempted validation using captive wobbegongs, and reported that the periodicity of vertebral band formation in captive animals did not support a synchronous annual pattern. Yet, captive growth rates matched those predicted when an annual band pattern was assumed for wild caught individuals. Similarly, the periodicity of band deposition in captive Japanese wobbegongs (*Orectolobus japonicus*) showed that a growth band usually formed annually in spring, but that the formation time sometimes varied with growth of the vertebral centrum rather than with time (Tanaka, 1990). In both of these studies, validation of the periodicity of the growth band formation was undertaken on captive sharks only and validation in wild sharks remains un-assessed.

In the present study, growth parameters of three sympatric species of wobbegongs were estimated by fitting growth models to length-at-age data obtained using thin cut sections of vertebrae. Growth parameters are compared using observed lengths-at-age and three back-calculated equations, and applied to four different growth models. Validation and verification of the periodicity of growth band deposition was also attempted using edge analyses, physical measurement of captive sharks, and chemical marking of captive and wild specimens.

2. Materials and methods

2.1. Collection of samples

Vertebrae were collected from *O. ornatus*, *O. maculatus*, and *O. halei* caught by hook and line fishing vessels, or captured using hand nets while scuba diving or free diving. Sharks were collected over the period June 2003–December 2007, at seven locations in Qld and NSW (Moreton Bay, Nambucca Heads, Port Stephens, Newcastle, Terrigal, Sydney and Eden) (Fig. 1).

One to four vertebrae were taken from the post-cranium region (vertebral numbers 1–4) of each specimen. Removal of vertebrae



Fig. 1. Map of (a) Australia and (b) locations where wobbegongs (family: *Orectolobidae*) were collected.

anterior to the first dorsal fin (i.e. the largest vertebrae) was not done because it lowers the carcass market value. Each shark was identified to species (Huveneers, 2006) and sex was determined by the presence of claspers in males. Before dissection, total length (TL) was measured using straight-line stretched measurements (Francis, 2006) to the nearest 1 mm. Vertebrae were stored on ice before being placed in a freezer at -20°C until further analysis.

2.2. Vertebral preparation

Vertebrae were thawed, excess tissue was removed, and individual centra separated using a knife before immersion in 5% sodium hypochlorite solution to remove any remaining flesh. Soaking time varied with vertebral size and ranged from 30 min for adult *O. ornatus* (ca. 10 mm in diameter) to two immersions of 1 h each for adult *O. halei* (ca. 25 mm in diameter). Excessive soaking was avoided because it tends to dissolve the centra and makes the articulating surfaces brittle and crumbly (Francis and Ó Maolagáin, 2000). Vertebrae were then placed in fresh running tap water for at least 30 min or left soaking overnight. Cleaned vertebrae were stored in a -20°C freezer until sectioned for age determination.

Numerous techniques have been used to enhance the visibility of growth bands in chondrichthyan vertebrae. The success of each technique is species-dependent and slight modifications of the method can enhance results (Goldman, 2005). Therefore, several techniques were tested on wobbegong vertebrae to identify a method for elucidating the clearest growth bands on whole vertebrae and thin cut sections. Alizarin red (Gruber and Stout, 1983; LaMarca, 1966) and crystal violet (Johnson, 1979; Schwartz, 1983) were used to stain whole and sectioned vertebrae, and a pencil method was also tested on whole vertebrae (Carlson and Parsons, 1997; Parsons, 1983, 1985). Neither staining nor the use of the pencil improved the clarity of growth bands. Thus, vertebrae were instead prepared as follows.

Prior to embedding in clear polyester casting resin cleaned vertebrae were oven dried at 55°C . A thin layer of resin was poured into a silicon mould and left to partially cure for ca. 30–60 min. An individual centrum was subsequently placed on the tacky surface and further resin was then poured into the mould until the centrum was completely covered. Blocks were left at room temperature for about 1 h and were then placed in an oven at 55°C until polymerization of the resin was complete. Sagittal sections were made perpendicular to the greatest centrum diameter, using a Gemmasta lapidary saw fitted with a diamond-impregnated blade. Up to five sections of ca. 350 μm thick were taken, ensuring that the focus of the centrum was included. The two best sections were rinsed in water and cleaned with alcohol prior to mounting onto a glass slide,

with a cover-slip placed over the section to avoid damage during handling and storage.

2.3. Reading of growth bands

Thin sections were viewed under a dissecting microscope (Olympus SZH) with a 0.75× lens connected to a Sony camera (DFW-X700). Images were visualized on an iMac computer (Apple, Cupertino, CA, USA) using the BTVPro software Version 5.4.1 (Ben Software, London, England). Thin sections were illuminated with transmitted light. A growth band was defined as a narrow, translucent band.

Growth bands of each section were counted, by two different readers on two occasions, after being chosen at random and without knowledge of the size or sex of the specimen. When present, notches along the outside edge of the *corpus calcareum* helped counts by providing an additional ageing feature (Goldman, 2005). The angle change on the centrum face, a result of the difference between fast intra-uterine and slower post-natal growth (Walter and Ebert, 1991), was considered as the birth mark and assigned as growth band 0. Any band observed before this birth mark was defined as a pre-birth mark (Allen and Wintner, 2002).

Each processed vertebra was assigned a 'readability score' of 1–6 based on the degree of difficulty in interpreting the arrangement of the vertebral bands (Officer et al., 1996). Vertebral sections with readability score of 3 or greater were excluded from further analysis. Any vertebra yielding an age estimate that differed by more than two bands between the two counts by CH (more experienced reader) was re-examined by both readers jointly, and age was assigned by consensus between both readers. For those age estimates that differed by 2 years or less, the mean value of the two estimates was assigned and used in growth models (Cotton et al., 2011). If the mean was between half a band count, the section was randomly assigned either reading.

The radius of each vertebra was measured on the *corpus calcareum* along a straight line through the focus of each vertebra with the 'Optimate' software (version 6.5) (Goldman, 2005; Goldman et al., 2006). Vertebral radius (VR) was plotted against wobbegong TL and tested for a linear relationship.

2.4. Precision and accuracy

Count reproducibility, as indicated by within and between reader variability, was determined by calculating an index of average percent error (IAPE) (Beamish and Fournier, 1981; Campana et al., 1995), the coefficient of variation (CV) (Campana et al., 1995; Chang, 1982), percent agreement (Cailliet, 1990), and age-bias plots of band counts (Campana et al., 1995).

2.5. Validation of using post-cranial vertebrae

Twenty-one *O. ornatus*, spanning the TL range of the sharks collected, were used to test the adequacy of using vertebrae from the post-cranial region. Growth band counts from post-cranial vertebrae were compared using a paired *t*-test to counts from the largest vertebrae, located in proximity to the first dorsal fin. Growth band counts from these regions were also compared to counts from the pre-caudal region to assess consistency of growth band formation throughout the vertebral column.

2.6. Models

A multi-model inference (MMI) information theoretical approach was used to determine the most appropriate growth model for each species (Burnham and Anderson, 2002; Katsanevakis and Maravelias, 2008; Thorson and Simpfendorfer,

2009). An *a priori* set of four candidate models were fitted to the length-at-age data. The candidate set consisted of: the traditional three-parameter von Bertalanffy growth model (VBGM – von Bertalanffy, 1938); a two-parameter modified form of the VBGM forced through the length-at-birth (L_0) (2P VBGM – Fabens, 1965), in the 2P VBGM, L_0 was estimated using the largest near-term embryo in Huveneers et al. (2011); the two-parameter Gompertz growth model (2P Gompertz – Bishop et al., 2006; Gompertz, 1825; Ricker, 1975), and the logistic model (Ricker, 1979).

Due to the small sample size of pup and juvenile wobbegongs, back-calculated estimates of length at previous age were calculated using three different equations. The best back-calculated equation was determined by comparing observed and back-calculated lengths-at-age. Observed lengths-at-age data, and a combination of back-calculated lengths and sample data were each separately fitted to the three growth models and the resulting parameter estimated compared.

Back-calculated lengths were not calculated using regression methodologies as they can overestimate fish length at capture (Francis, 1990). Instead, three proportion-based back-calculation methods were investigated and compared to examine the statistical and biological accuracy of back-calculations relative to vertebral sample data (Goldman et al., 2006): the Dahl–Lea direct proportions method (Carlander, 1969), the linear-modified Dahl–Lea method (Francis, 1990), and the quadratic-modified Dahl–Lea method (Francis, 1990). The size-at-birth-modified Fraser–Lee equation was not used because it does not follow back-calculation hypotheses and is based on a misuse of linear regression (Francis, 1990).

Model parameters were estimated by non-linear least-squares regression in SPSS v.18 and model performance evaluated using Akaike's (1973) Information Criterion (AIC). The small sample bias-adjustment form of AIC was calculated as $AIC_c = AIC + 2K(K+1)(n-K-1)^{-1}$, where K is the total number of estimated parameters+1 for variance (σ^2), n is the samples size, and $AIC = n \log(\sigma^2) + 2K$. Variance was calculated as $\sigma^2 = RSS/n$ where RSS is the sum of the squared residuals. The best model was the one with the lowest AIC_c value. For model comparisons, the delta AIC (ΔAIC) and Akaike weights (w_i) were calculated (Burnham and Anderson, 2002). The ΔAIC is a measure of each model relative to the best model and is calculated as $\Delta AIC = AIC_i - \min AIC$, where AIC_i is AIC value for model i and $\min AIC$ is the AIC value of the best model. Models with ΔAIC of 0–2 had substantial support, while models with ΔAIC of 4–7 had considerably less support and models with $\Delta AIC > 10$ had essentially no support. Akaike weights (w_i) represent the probability of choosing the correct model from the set of candidate models, and are calculated as:

$$w_i = \frac{\exp(-(\Delta AIC/2))}{\sum_{i=1}^R \exp(-(\Delta AIC/2))}$$

where R is the number of candidate models. Once the best model was determined, a likelihood ratio test (Cerrato, 1990; Kimura, 1980) was used to test for differences between growth curves of males and females (Haddon, 2001).

2.7. Validation and verification

Several methods were compared to verify and validate the periodicity of growth band deposition: edge and marginal increment analyses, growth rate of captive sharks, and the chemical marking of captive and wild sharks (Cailliet and Goldman, 2004; Goldman, 2005).

2.7.1. Edge and marginal increment analyses

The periodicity of band formation was evaluated using two methods of centrum edge analysis. The last deposited band was classified as translucent or opaque and related to the month of capture (Kusher et al., 1992). Marginal increment analysis was undertaken by measuring the distance from the last band to the edge of the centrum as a proportion of the distance between the last and the penultimate bands for vertebrae that have clear band patterns and undamaged centrum edges (Branstetter and Stiles, 1987; Cailliet, 1990):

$$\text{MIR} = \frac{\text{VR} - \text{GB}_n}{\text{GB}_n - \text{GB}_{n-1}}$$

where MIR is the marginal increment ratio, VR is the vertebral radius, GB_n the distance from the centra to the last band and GB_{n-1} is the distance from the centra to the penultimate band. Temporal variation of MIR was examined using a Kruskal–Wallis test.

2.7.2. Growth rate of captive wobbegongs

To estimate growth rates in captivity, specimens of all three species were maintained for up to 88 months in indoor aquaria of 1000 to 2.2 Ml capacity depending on the size and number of wobbegongs held. Wobbegongs were held in aquaria located in Sydney, NSW, equipped with a flow-through system, and were therefore subjected to the natural variation in water temperature (about 16–22 °C). The photoperiod in the aquaria was artificially mimicking the natural, diel cycle. Sharks were fed a combination of whiting, pilchards, yellowtails, trevally, squid and octopus at least twice a week and up to six days/week. Sharks were individually recognizable by dorsal skin patterns, such as spot position and shape of saddles, and were identified using photo-identification for the purpose of repeat, monthly measurement of TL to the nearest 1 mm. Sharks of similar TL and born or captured together were grouped together.

2.7.3. Chemical marking of wobbegongs

Captive sharks that could be sacrificed were also injected with oxytetracycline (OTC, Ilium Oxytet-200 L.A.). Sharks were measured, weighed and injected with 25 mg/kg of OTC before being released in the aquarium (McFarlane and Beamish, 1987). Sharks were later sacrificed by pithing and were measured and dissected to collect vertebrae using the methods described above. Between December 2004 and October 2007, sharks were also labelled with fluorochrome dye in the natural habitat off Amity Point, North Stradbroke Island, Queensland. Wobbegongs (*O. maculatus*) caught by hand-net while free-diving were tagged on either side of the dorsal fin with individually coded plastic tipped dart tags (PDA) (Hallprint Pty Ltd., South Australia), measured to the nearest 1 mm and injected intra-muscularly with calcein (5–10 mg/kg in sterile 0.9% NaCl, pH 7.5) (Gelsleichter et al., 1997). In October 2007, one of the tagged *O. maculatus* was recaptured and, following euthanasia, a section of the vertebral column was excised and processed as described earlier. The calcein stained sagittal section was viewed under UV light on a compound microscope (Olympus BH2, Japan). The number of growth bands following OTC and calcein marks was counted and the temporal periodicity of the formation of growth bands was estimated by comparing known dates of chemical injection with the number of growth bands counted after the chemical mark.

2.7.4. Comparison of wild and captive growth

The growth rates from captive and wild wobbegongs were compared by generating two growth curves using the best growth model (logistic model for *O. ornatus* and *O. halei*, and VBGM for *O. maculatus*) and assuming that growth bands represented six

Table 1

Number of wobbegongs sampled and number of sections with a readability score of 3 or greater, and used in analyses.

Species	Sex	Sharks sampled	Sections
<i>O. ornatus</i>	Male	123	97
	Female	150	104
	Unknown	2	1
	Total	275	201
<i>O. maculatus</i>	Male	121	85
	Female	108	80
	Unknown	3	
	Total	232	165
<i>O. halei</i>	Male	114	87
	Female	131	97
	Unknown	8	4
	Total	253	188
Species combined		760	554

months of growth or one year of growth. Growth rates of captive wobbegongs were then compared with each growth curve to determine the most appropriate estimate of the periodicity of growth band formation. The age of each animal at its initial length (t) was estimated by solving the known initial length for the two growth scenarios. The growth rate of each animal was then produced by plotting the known observed final length against t plus the period of captivity and fitting a straight line between each data point. This method was previously used by Chidlow et al. (2007) to investigate the periodicity of growth band deposition in *O. hutchinsi*.

3. Results

3.1. Collection of samples

Vertebrae from 760 wobbegongs (275 *O. ornatus*, 232 *O. maculatus*, and 253 *O. halei*) were excised and processed to count growth bands. As a result of poor readability or count difference ≥ 3 , thin sections from 201 (76.4%) *O. ornatus*, 165 (71.1%) *O. maculatus*, and 188 (74.3%) *O. halei* were retained and used for age and growth analysis (Table 1).

All three species had a significant linear relationship between VR of the anterior, immediately post-cranial vertebrae and shark TL, indicating that these vertebrae were a suitable structure for age determination (Fig. 2 and Table 2). The mean radius of the birth band was 1.2 ± 0.01 mm (mean and s.e.), 1.4 ± 0.02 mm and 1.7 ± 0.02 mm for *O. ornatus*, *O. maculatus* and *O. halei*, respectively. Similarly, the mean vertebral radius of near-term embryos was 1.1, 1.2, and 1.4 mm, for *O. ornatus*, *O. maculatus*, and *O. halei*, respectively, indicating that the birth band was identified correctly. The maximum number of growth bands observed was 20, 22, and 27 for *O. ornatus*, *O. maculatus* and *O. halei*, respectively.

Table 2

Relationship between vertebral radius and wobbegong total length. Values of parameters and statistical quantities for the equation $\text{TL} = a' + b' \text{VR}$; VR is vertebral radius; TL is shark total length; a' and b' are parameters; n is sample size; r^2 is square of regression correlation coefficient; rmse is root mean square error; and P is the probability of statistical significance.

Species	a' (\pm s.e.)	b' (\pm s.e.)	n	r^2	rmse	P
<i>O. ornatus</i>	229.6 (38.1)	131.4 (8.4)	202	0.56	5131	<0.001
<i>O. maculatus</i>	401.7 (52.4)	124.9 (7.9)	156	0.62	10,967	<0.001
<i>O. halei</i>	191.9 (53.2)	165.8 (6.2)	188	0.79	14,342	<0.001

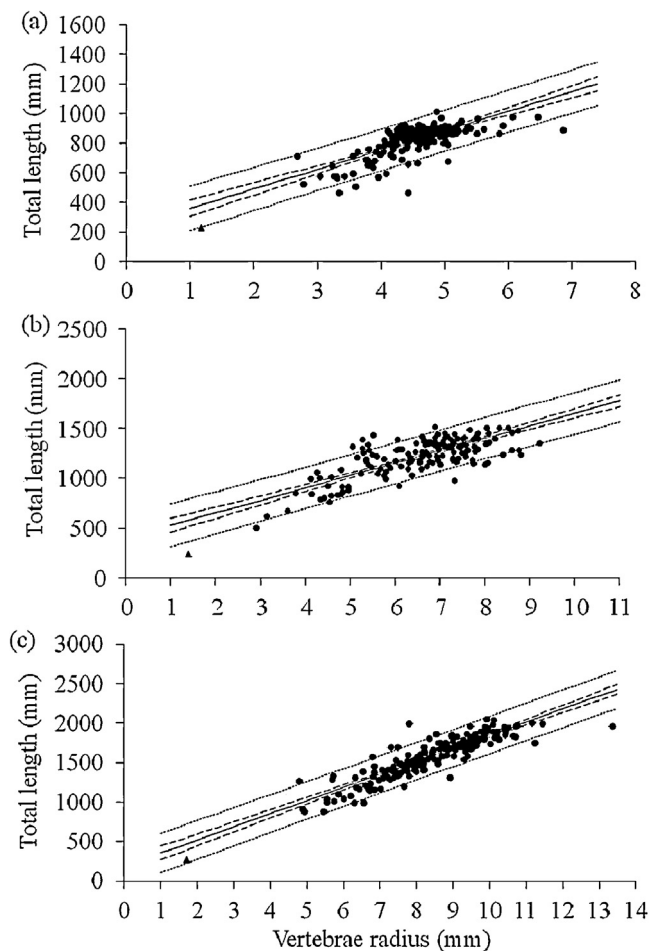


Fig. 2. Relationship between vertebral radius and wobbegong total length. Shark total length (—), 95% confidence limits (---), 95% prediction intervals (····), raw data (●), and (▲) near-term embryos are plotted against vertebral radius for (a) *O. ornatus*, (b) *O. maculatus*, and (c) *O. halei*.

3.2. Precision and accuracy

Average percent error and CV of all three species were sufficiently low to indicate reproducible counts and high precision between the two readings (Campana, 2001).

Within reader bias: all APEs were lower than 4%, whereas all CVs were less than 6%. Percent agreement did not indicate any strong biases between the two readings with 91.9%, 91.3% and 89.9% of the counts similar within \pm two growth bands for *O. ornatus*, *O. maculatus* and *O. halei*, respectively. Similarly, within reader bias was not observed in age-bias plots (Fig. 3).

Between reader bias: the between reader precision was slightly less than within reader precision. All APEs were lower than 6%, whereas all CVs were less than 7%. *Orectolobus maculatus* had the highest APE and CV. Percent agreement did not indicate any strong biases between the two readers with 89.6%, 81.1%, and 83.1% of the counts similar within \pm two growth bands for *O. ornatus*, *O. maculatus* and *O. halei*, respectively. In *O. ornatus* and *O. maculatus*, the growth band counts were slightly underestimated in the second reader's final count (Reader 2), compared with the more experienced first reader. These biases were observed mostly in older individuals after 15 and 18 growth bands in *O. ornatus* and *O. maculatus*, respectively (Fig. 3). In *O. halei*, the bias in older individuals was not observed, but growth band counts were slightly overestimated by Reader 2 in younger individuals.

3.3. Validation of using post-cranial vertebrae

O. ornatus ranged 525–919 mm TL and had 5–13 growth bands. There was no significant difference in the number of growth bands in vertebrae from the post-cranial region and from the region ventral to the first dorsal fin within individual sharks (paired t -test: $t_{21} = 1.55$, $P > 0.05$). The number of observable growth bands in vertebrae in these two regions was, however, greater than the number of growth bands in vertebrae from the pre-caudal region (paired t -test: $t_{20} = 5.75$, $P < 0.0001$ and $t_{22} = 2.83$, $P < 0.01$), suggesting that pre-caudal vertebrae are not suitable for age and growth estimation in wobbegongs.

3.4. Models

Although the linear regression gave a significant fit to the VR–TL data, it was important to compare the mean back-calculated results from each back-calculated method with our mean sample TL data to see which method provided better biological accuracy for modelling growth (Goldman et al., 2006). Back-calculated lengths obtained from the Dahl–Lea linear proportion method were similar to the mean observed lengths-at-age for large juvenile and adult wobbegongs, but were higher than the mean observed lengths-at-age for the young to small juveniles in all species (according to the sizes-at-maturity in Huveneers et al., 2007). The back-calculated lengths obtained from the Dahl–Lea linear proportion method were also higher than the other back-calculated lengths in young sharks and overestimated the size-at-birth of all three species, with the largest overestimate occurring in *O. maculatus*. The quadratic-modified Dahl–Lea method was more variable. While it gave a good length-at-age estimation for adult and large juvenile *O. ornatus*, it slightly underestimated its size-at-birth as well as the entire range of *O. halei* (Fig. 4). It, however, provided back-calculated lengths close to the mean observed lengths in *O. maculatus*. The back-calculated lengths obtained using the Dahl–Lea direct proportion method gave estimates closest to mean observed lengths-at-age and size-at-birth for all three species, and was therefore used when combining observed and back-calculated lengths-at-age.

For all three species, the residual sum of squares (RSS) and AIC_c were lower for the model fitting the observed lengths-at-age than for the models fitting the combined observed and back-calculated lengths-at-age. Therefore, models using a combination of observed and back-calculated lengths-at-age were not investigated further, and models discussed further refer to models fitted to observed lengths-at-age only. The logistic model overestimated size-at-birth in *O. maculatus*. By contrast, the VBGM slightly underestimated size-at-birth of *O. ornatus* and greatly underestimated size-at-birth of *O. halei* (Table 3 and Fig. 5). Growth curves obtained from the VBGM and 2P VBGM had higher L_∞ and smaller k than curves obtained from the 2P Gompertz and the logistic models. The 2P VBGM had the largest estimates of L_∞ and smallest estimates of k for *O. ornatus* and *O. halei*, and second largest and smallest estimates for L_∞ and k , respectively for *O. maculatus*. The logistic model estimated the smallest L_∞ and the largest k estimates for *O. ornatus* and *O. halei* compared to the other models. For *O. ornatus*, the maximum TL sampled was similar to the L_∞ estimated by the VBGM and the 2P VBGM. However, the 2P Gompertz and logistic models underestimated L_∞ for this species, while the 2P VBGM estimated L_∞ larger than sampled animals (Table 3 and Fig. 5). According to the AIC_c and w_i , the logistic growth function fitted the observed lengths-at-age of *O. ornatus* and *O. halei* best, while the VBGM fitted observed lengths-at-age of *O. maculatus* best. Based on these models, there were no statistical differences between males and females with all three species ($\chi^2 = 7.49$, 7.29, 7.19 for *O. ornatus*, *O. maculatus*, and *O. halei*, respectively; $P > 0.05$ for all species).

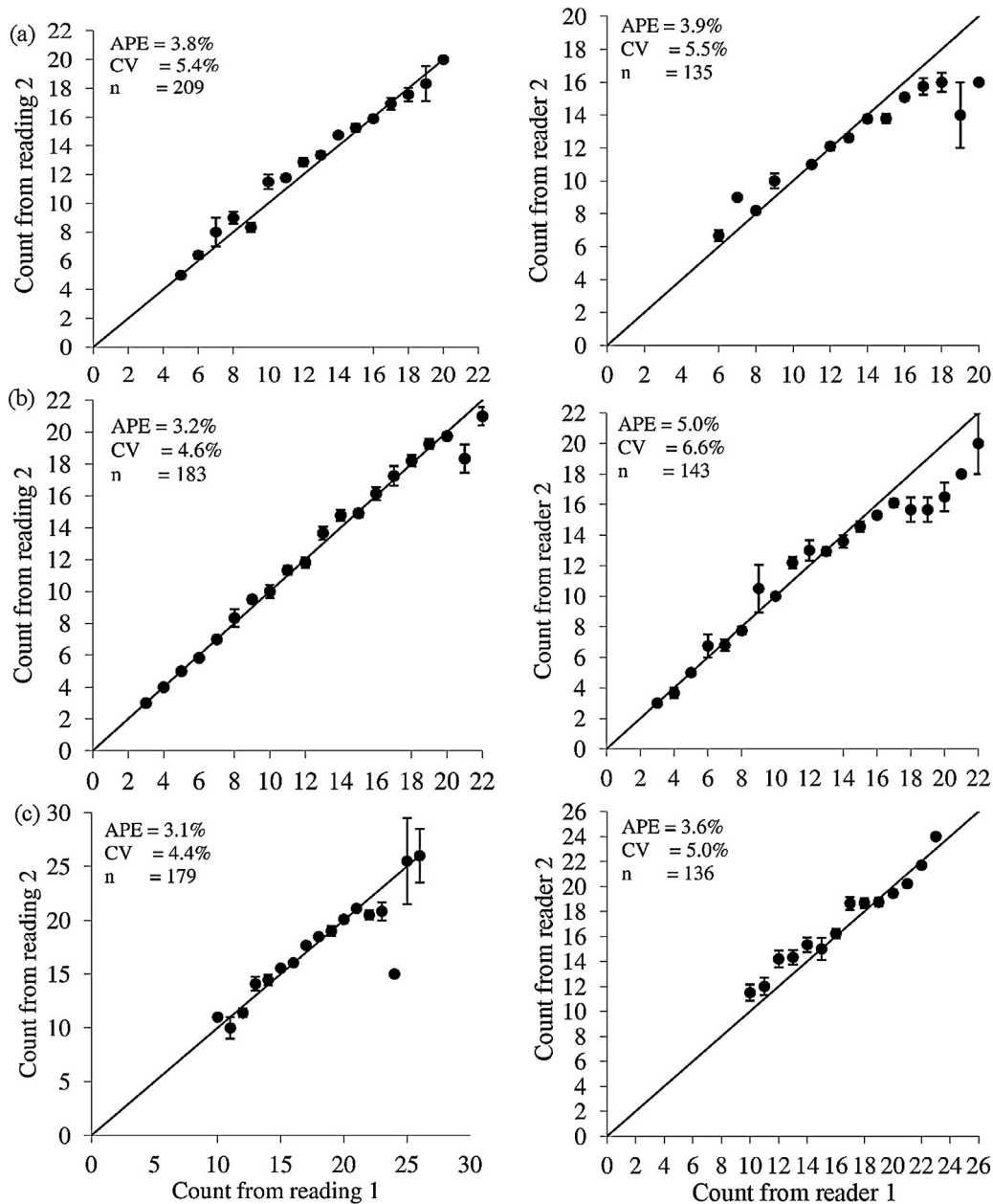


Fig. 3. Age-bias plots of band counts for wobbegongs in eastern Australia. Left figures represent within-reader bias, right figures represent between-reader bias. (●) mean; bars, standard error; APE, average percent error; CV, coefficient of variation; n, number of thin cut sections randomly sub-sampled to estimate within and between reader biases for (a) *Orectolobus ornatus*, (b) *O. maculatus*, and (c) *O. halei*.

3.5. Validation and verification

3.5.1. Edge and marginal increment analyses

Categorisation of growth bands at the edges of the vertebrae as opaque or translucent was difficult. No clear pattern in the last growth band was detected for any of the three species (Fig. 6).

Departure of homogeneity of variance for marginal increment ratio (MIR) calculation was significant for *O. ornatus* (Levene test: $F_{df=8,168} = 2.7$, $P < 0.01$) and *O. maculatus* (Levene test: $F_{df=9,100} = 4.0$, $P < 0.001$), but not for *O. halei* (Levene test: $F_{df=11,169} = 1.2$, $P > 0.05$). MIR differed significantly among months (ANOVA: $F_{df=11,169} = 3.00$, $P < 0.01$) for *O. halei*, but not for *O. ornatus* (Kruskal–Wallis test: $\chi^2_{df=10} = 15.12$, $P > 0.05$) nor for *O. maculatus* (Kruskal–Wallis test: $\chi^2_{df=10} = 6.83$, $P > 0.05$). No clear pattern was detected in the mean monthly MIR for *O. ornatus* or *O. maculatus*. However, in *O. halei* the

mean MIR was highest during March–April and September, and lowest during May and November.

3.5.2. Growth rate of captive wobbegongs (Table 4)

Three *O. ornatus*, 31 *O. maculatus* and 6 *O. halei* were kept in captivity between 6 and 88 months (0.5–7.3 years). All sharks in captivity showed a decline in growth rate with increasing TL. The neonate *O. ornatus* grew ca. 350 mm in nearly two years averaging a monthly growth rate of 16 mm/month. However, the two mature *O. ornatus* with TL close to the reported maximum TL did not grow as fast and grew only ca. 2 mm/month. In *O. maculatus*, growth rate also decreased with increasing TL. Neonates of ca. 320 mm TL grew ca. 24 mm/month, whereas neonates of 410–450 mm TL grew ca. 17–20 mm/month as did two juvenile of ca. 570 mm TL. Five *O. maculatus* of ca. 660 mm TL grew ca. 8.5 mm/month, whereas a 1070 mm TL specimen only grew ca. 5 mm/month at the

Table 3

Growth parameters for *O. ornatus*, *O. maculatus*, and *O. halei* calculated from thin sections and a combination of back-calculated and observed lengths-at-age, and using the von Bertalanffy growth model (VBGM); the two-parameter modified form of the VBGM forced through the length-at-birth (2P VBGM), the two-parameters Gompertz growth model (2P Gompertz), and the logistic model, L_{∞} is theoretical maximum total length; k is growth coefficient; t_0 is theoretical age at zero length; α is time at which the absolute rate of increase in length begins to decrease, or the inflection point of the curve; RSS is residual sum of squares; AIC_c is the small sample bias-adjustment form of Akaike Information Criterion; and w_i is Akaike's weight.

Species	Sample	VBGM			2P VBGM					2P Gompertz					Logistic								
		L_{∞}	k	t_0	RSS	AIC _c	w_i	L_{∞}	k	RSS	AIC _c	w_i	L_{∞}	k	RSS	AIC _c	w_i	L_{∞}	k	α	RSS	AIC _c	w_i
<i>O. ornatus</i>	Samples only	109.88	0.10	-1.09	3763	1180	0.01	119.26	0.07	3803	1182	0.00	102.21	0.15	3724	1174	0.27	99.86	0.19	5.06	3688	1172	0.71
	Back-calculated	125.02	0.07	-2.33	4178	1301	0.00	122.56	0.07	4143	1296	0.00	102.81	0.15	3994	1280	0.24	98.71	0.20	5.25	3956	1277	0.76
<i>O. maculatus</i>	Samples only	175.60	0.07	-2.84	9934	1340	0.69	163.00	0.09	10,119	1344	0.09	144.10	0.19	11,715	1391	0.00	155.27	0.16	5.09	10,005	1341	0.22
	Back-calculated	171.74	0.08	-2.41	9969	1353	0.85	162.82	0.10	10,135	1357	0.15	145.17	0.19	11,464	1397	0.00	152.00	0.17	5.08	10,304	1364	0.00
<i>O. halei</i>	Samples only	240.07	0.09	4.66	24,999	1806	0.36	1,681,728	0.00	29,384	1863	0.00	275.08	0.08	26,412	1826	0.00	212.77	0.20	10.96	24,918	1804	0.64
	Back-calculated	717.632	0.00	-4.79	32,032	2101	0.00	1,385,866	0.00	34,301	2128	0.00	271.40	0.09	29,711	2068	0.00	257.16	0.12	12.69	27,175	2033	1.00

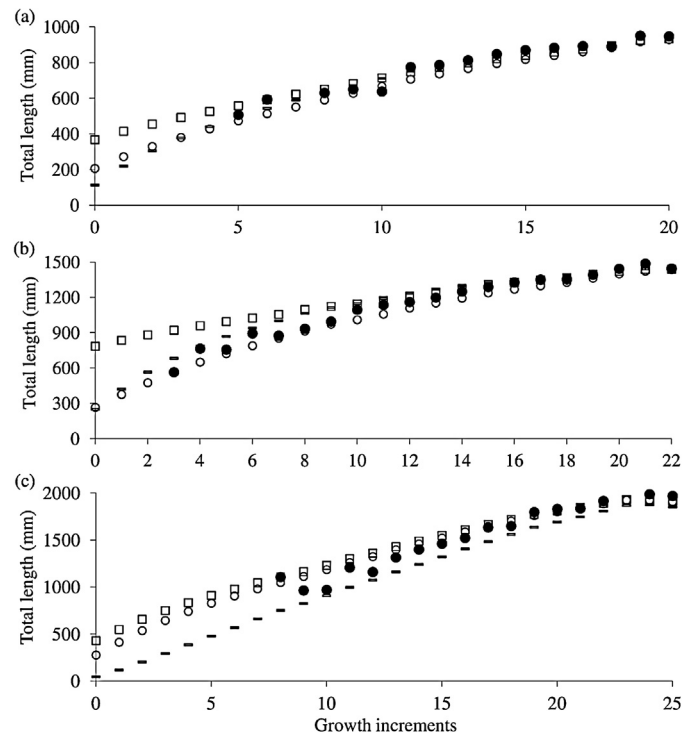


Fig. 4. Back-calculated lengths-at-age estimates for wobbegongs in NSW. Back-calculated length-at-age using measurement obtained on thin sections and the Dahl-Lea linear (○), the Dahl-Lea linear (□), and the Dahl-Lea quadratic (—) compared to sample mean length-at-age (●) for (a) *Orectolobus ornatus*, (b) *O. maculatus*, and (c) *O. halei*.

slowest growth rates recorded. Five *ca.* 400 mm TL only grew *ca.* 9 mm/month, which is a slow growth for wobbegongs of this size compared to the other recorded growth in this study. These individuals were, however, recorded for more than seven years during which growth could have been initially fast prior to slowing down with age. Neonate *O. halei* grew *ca.* 512 mm in 2.5 years, averaging a monthly growth rate of 17 mm/month, which was similar to that of neonate *O. ornatus*.

3.5.3. Chemical marking of captive wobbegongs (Table 4)

Although all sharks injected with OTC showed at least one fluorescent band when viewed under ultraviolet light, multiple injections of OTC did not always correspond to multiple fluorescent lines. One neonate (231 mm TL) and two mature male (905–945 mm TL) *O. ornatus* were injected with OTC. Date of birth of the neonate was known because it was removed from a female uterus as near-term embryo. The neonate shark was injected with OTC at 5 and 20 months of age prior to being euthanized at 2.5 years of age. Four growth bands were observed after the birth band, but only one opaque band was seen after the OTC mark. Both adult *O. ornatus* were injected one year before being euthanized. Fifteen and 16 growth bands were observed after the birth band, and a narrow opaque band was observed after the OTC mark.

Five captive *O. maculatus* (647–694 mm TL) were injected with OTC six months prior to analysis of their vertebrae. In all five sharks, an opaque band was present between the OTC mark and margin of the vertebra, although some sharks had an opaque and a translucent band present. These sharks had 5–6 growth bands after the birth band, but poor readability of these vertebrae made it difficult to categorize growth bands with certainty. Another *O. maculatus* (571 mm TL) was injected with OTC on two occasions: at one year and at four months prior to being euthanized. Seven growth bands were observed after the birth band, but only one OTC

Table 4

Details of growth rate and fluorochrome marking of captive and wild wobbegongs group is sharks of similar total length (TL), and born or captured together; *n* is sample size; TL is total length at which sharks were born or captured (mm); Growth is total growth during study (mm); period is the number of months between first and last measurements; monthly ave growth is average monthly growth (mm/month); yearly ave growth is average yearly growth (mm/year); marked period is the number of months between fluorochrome injection and euthanasia, several numbers indicate that sharks were injected several times; no. of fluorescent growth band is the number of fluorescent band observed; additional growth band is the number of growth bands distal to the fluorescent mark. When more than one fluorescent band was observed, the number of growth bands after each fluorescent band is provided.

Species	Group	<i>n</i>	TL mean (s.e.)	Growth mean (s.e.)	Period (month)	Monthly ave growth mean (s.e.)	Yearly ave growth mean (s.e.)	Total no of growth band	Marked period (month)	No of fluorescent band	Additional growth band
<i>O. ornatus</i>	1	1	231	352	22	16	192	4	20 and 5	1	1 opaque band
	2	2	925 (20)	20.5 (5)	11	1.9 (0.5)	22.4 (6.0)	15–16	12	1	1 opaque band
<i>O. maculatus</i>	3	3	318.7 (8)	261.7 (6)	11	23.8 (0.5)	285.5 (6.3)		–	–	–
	4	6	355.0 (23)	845.0 (33)	65.7 (0.6)	12.9 (0.6)	154.7 (7.0)		–	–	–
	5	2	401.5 (12)	828.5 (47)	88	9.4 (0.5)	113.0 (6.5)		–	–	–
	6	1	414	430	24	17.9	215		–	–	–
	7	3	442.3 (25)	184.3 (11)	11	16.8 (1.0)	201.1 (12)		–	–	–
	8	2	445.5 (17)	309.5 (11)	15	20.6 (0.8)	247.6 (9.2)		–	–	–
	9	1	571	256	13	19.7	236.3	5–6	12 and 4	1	1 opaque band
	10	7	645.7 (15)	154.9 (5)	8	19.4 (0.7)	232.3 (8.6)		–	–	–
	11	5	663.8 (9)	55.4 (4)	6	8.5 (0.6)	102.3 (7.0)	7	6	1	1 band pair
	12	1	1070	380	69	5.5	66.3		–	–	–
	13	1	1022	1170	30	4.9	59.2	12	30 and 10	2	1 band pair/1 opaque
<i>O. halei</i>	14	6	262.3 (2)	512.7 (13)	30	17.1 (0.4)	205.1 (5.1)		–	–	–
	15	2	1433 (33)	^a	^a	^a	^a	15	18	1	2 band pairs

^a TL was not measured when sharks were euthanized.

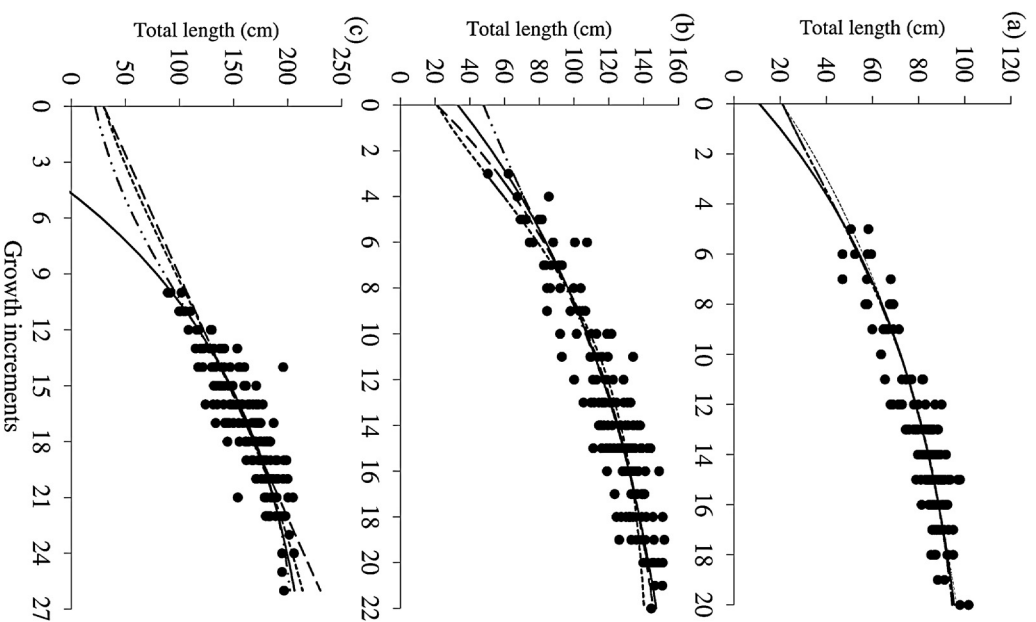


Fig. 5. Growth curves for wobbegongs in NSW. Growth curves generated from thin sections for (a) *Orectolobus ornatus*, (b) *O. maculatus*, and (c) *O. halei*. Growth curves were fitted to the observed lengths-at-age data using the von Bertalanffy growth model (—), the two-parameter von Bertalanffy growth model (---), the two-parameter Gompertz growth model (-.-), and the logistic growth model (-.-).

mark was discernable close to the edge of the vertebrae, followed by an opaque band.

Two immature *O. halei* (1160–1320 mm TL) were injected with OTC 18 months prior to being euthanized. In both individuals, 15 growth bands were observed after the birth band, the OTC mark was highly visible, and two growth bands (two opaque and two translucent bands) were observed to have been formed after the OTC mark.

3.5.4. Chemical marking of wild wobbegongs (Table 4)

Sixty-eight *O. maculatus* were double-tagged, chemically marked with the fluorochrome dye calcein, and released off Amity Point, North Stradbroke Island, Qld. Fifteen individuals were recaptured a total of 23 times with sharks recaptured up to three times. One 1022 mm TL individual initially captured and injected with calcein in April 2005 was recaptured and re-injected with calcein in December 2006. This individual was recaptured in October 2007, when it was euthanized. This specimen has 12 growth bands after the birth band, with two distinct fluorescent lines evident when viewed under UV light in the sagittally sectioned centra of this individual (Fig. 7). The prominent stain, visible under normal transmitted light, however, obscured the centrum's natural banding pattern, which made it difficult to discern the number of growth

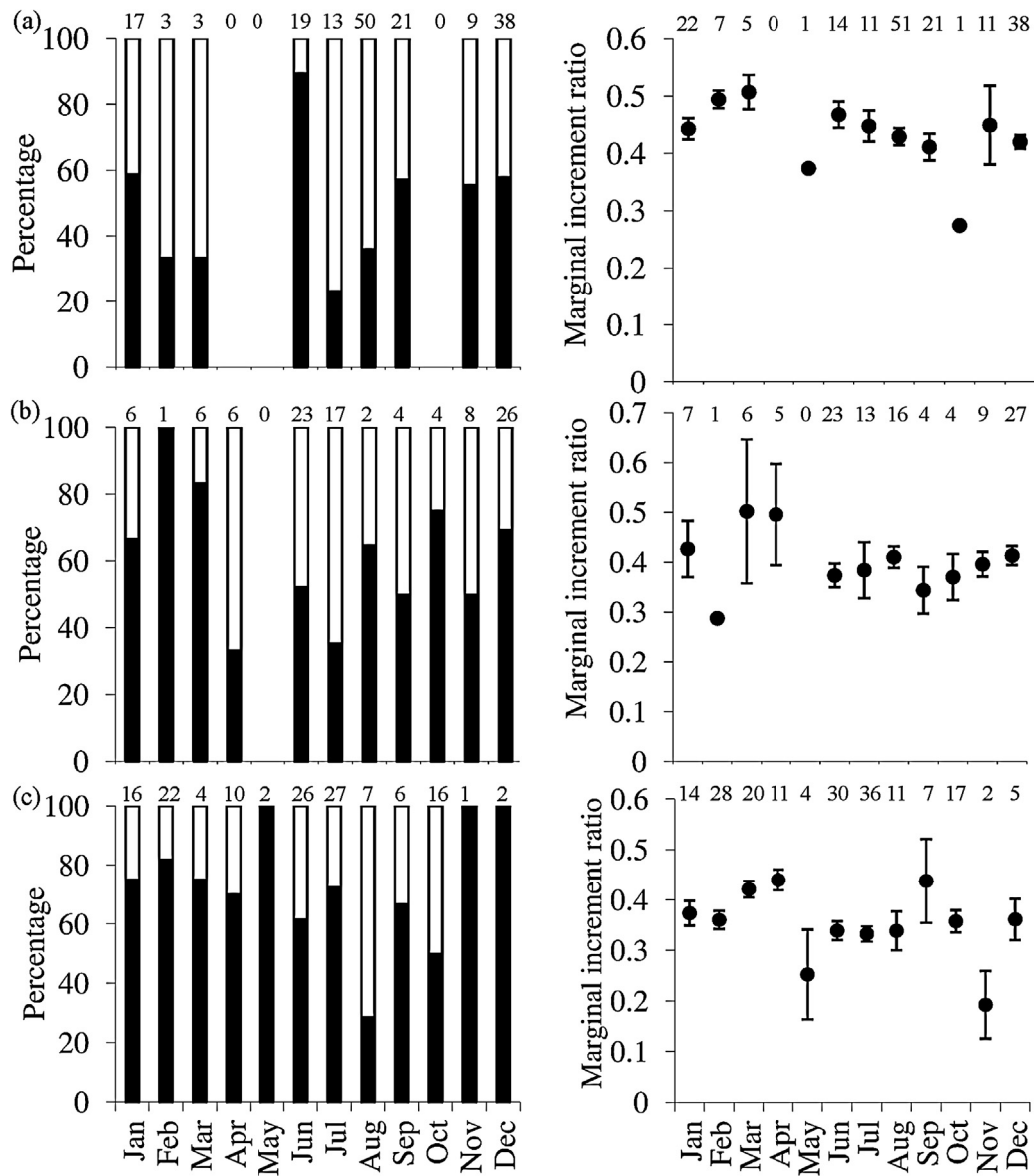


Figure 6. Monthly variation of vertebra edge and marginal increment ratio (MIR). Monthly frequency of translucent (□) and opaque bands (■) determined from and thin sections (left), and monthly variation of mean marginal increment ratio (right) for (a) *Orectolobus ornatus*, (b) *O. maculatus* and (c) *O. halei*. Sample size is indicated on top of the graphs; (●) mean monthly MIR; bars, standard error for monthly value.

bands present. Nevertheless, it appeared that one band pair was present between the first and second calcein marks and an opaque band was present after the second calcein stain (Fig. 7).

3.5.5. Comparison between wild and captive growth

For all three species of wobbegongs, captive growth rates were considerably higher than growth rates estimated for individuals from the natural habitat if it is assumed that one growth band is formed per year. Captive growth more closely matched wild growth if the assumption that two bands were formed per year was adopted (Fig. 8).

4. Discussion

Several authors have stressed the need to validate the periodicity of band deposition and absolute age for accurate age estimation (e.g. Beamish and McFarlane, 1983; Cailliet, 1990; Cailliet and Goldman, 2004). Various methods were attempted to verify and validate the periodicity of band deposition in wobbegongs. Neither

edge analysis nor MIR showed patterns indicating such periodicity for any of the three species examined. Although MIR differed significantly among months in *O. halei*, it did not provide a clear trend of periodicity in growth band formation (Lessa et al., 2004). The lack of periodicity observed might have been due to the difficulty in categorizing the edges of the *corpus calcareum*, and the small sample sizes in any particular month (Cailliet, 1990). To overcome the difficulty of measuring the width of the last growth band in older sharks (Allen and Wintner, 2002), and because MIR conducted over all age classes can be different when restricted to a single age (Campana, 2001), MIR calculations can be done on juveniles only (Jacobsen and Bennett, 2010; Neer et al., 2005). This was not feasible in this study due to the minimal numbers of neonates and small juveniles. Although MIRs are frequently used as validation techniques, it does not often result in periodicity being determined (e.g. Allen and Wintner, 2002; Carlson et al., 1999; Lessa et al., 2004; Santana and Lessa, 2004; Wintner et al., 2002) and chemical marking is frequently recommended to validate periodicity in growth band deposition.

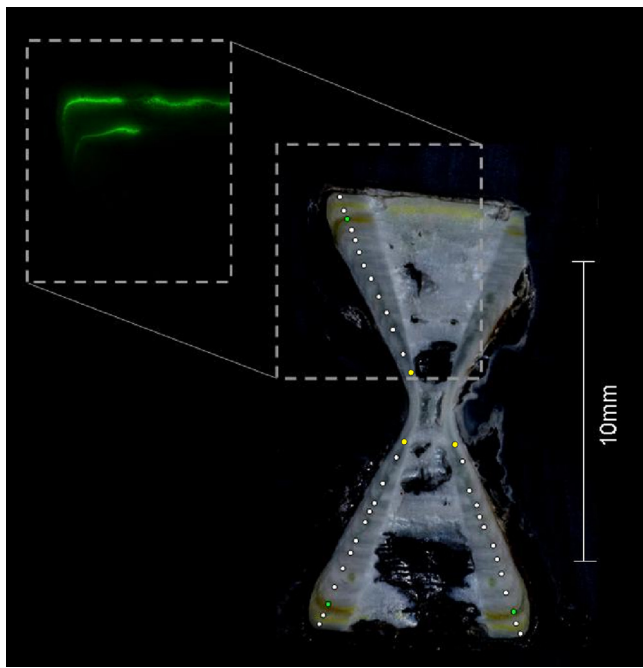


Fig. 7. Calcein markings on a vertebra from a 1022 mm TL *Orectolobus maculatus* recaptured after 30 months at liberty. Yellow dot represents birth band, white circles represent growth bands, green circles represent calcein marking. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Fluorochrome marking of vertebrae to try and assess growth rates was attempted in both captive and wild wobbegongs. The injected fluorochrome, while obvious in some sectioned vertebrae was not visible in others. The lack of fluorochrome marking in some individuals has previously been observed in other species (e.g., Kusher et al., 1992), and the reason for this was not clear. Normally, a fluorochrome label would bind to calcium ions and be deposited, along with the calcium, in tissues that are undergoing calcification, such as the margins of vertebral centra (Tanaka, 1990). A lack of labelling could be the result of technical issues such as a failure in delivery of the dye or application of an insufficient dose. Alternatively, the lack of a visible label could have a biological explanation. As sharks age, the rate of apposition of new calcified cartilage decreases, hence the growth bands are formed closer together near the edge of the centrum compared to adjacent to the birth band (Cailliet, 1990; MacNeil and Campana, 2002; Skomal and Natanson, 2003). A very low rate of apposition may result in a faint or non-visible fluorescent line. It is also possible that there was an absence of appositional growth at the time of label application, either due to natural variation in growth or due to a temporary halt in growth due to the stress of capture or handling. In the case of the captive sharks, handling might have also produced irregular growth band deposition or 'disturbance check marks', biasing periodicity estimates (Walker et al., 1995). In summary, oxytetracycline and calcein labelling of vertebrae did not provide conclusive evidence for annual growth band deposition.

The number of growth bands deposited in small individuals suggested a higher than annual rate of deposition. In contrast, large individuals fitted the assumption of annual growth band deposition. This finding is consistent with previous age and growth

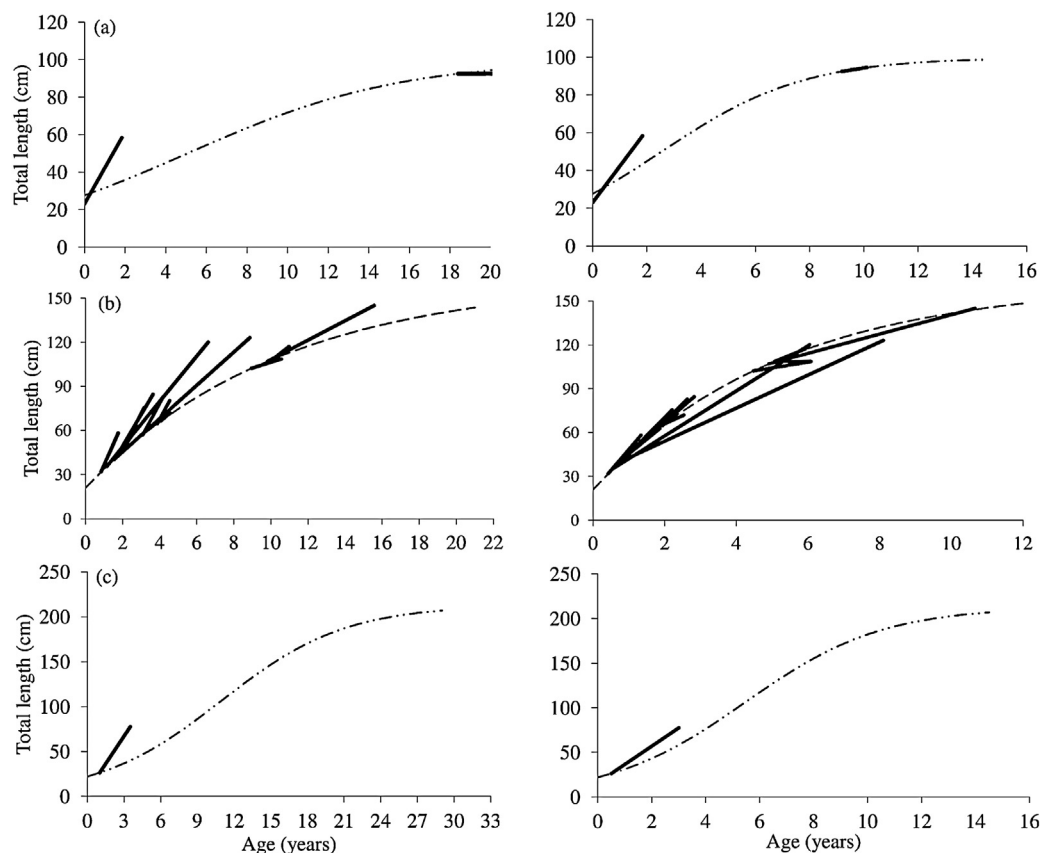


Fig. 8. Comparison of captive and wild growth for (a) *Orectolobus ornatus*, (b) *O. maculatus*, and (c) *O. halei*. Dashed lines are growth curves from vertebral age determination assuming (left) one band per year and (right) two bands per year, black lines are total length growth rates of captive wobbegongs plotted on the relevant portion of the growth curve.

studies of orectolobids that were unable to validate annual band deposition, and suggested that growth band deposition in wobbegongs is likely to be linked to somatic growth (Chidlow et al., 2007; Tanaka, 1990). Although growth band deposition in *O. japonicus* seemed to be annual, the OTC injection of captive sharks did not clearly show periodicity in growth band deposition (Tanaka, 1990). In *O. hutchinsi*, ontogenetic variation in growth band deposition was found, with neonates producing up to three growth bands in their first year, whereas adults form less than one band per year, averaging growth band periodicity to one per year (Chidlow et al., 2007). Including the current study, five orectolobid species appear not to deposit growth bands on an annual basis, as suggested for most other chondrichthyan species (Cailliet, 1990; Ferreira and Vooren, 1991; Goldman, 2005).

The lack of temporally predictable growth band deposition has also been observed in the Pacific angel shark (Order Squatiniformes: *Squatina californica*), in which bands also appear related to somatic growth (Natanson and Cailliet, 1990). Although phylogenetically distant Orectolobidae and Squatinidae share similar biological and ecological characteristics including occupancy of benthic habitats (Compagno, 2001) and multi-year reproductive cycles (Colonello et al., 2007; Huveneers et al., 2007) and such factors, related to their life-histories, may influence vertebral growth. Biannual growth band deposition has been suggested in some chondrichthyan species: *Sphyrna lewini* (Chen et al., 1990; Tolentino et al., 2008; Tolentino and Mendoza, 2001), *Isurus oxyrinchus* (Pratt and Casey, 1983), *Carcharias taurus* (Branstetter and Musick, 1994), and *Cetorhinus maximus* (Parker and Stott, 1965). Validation and verification methods have, since, provided evidence of annual or somatic growth band deposition in all four species (Bishop et al., 2006; Branstetter, 1987; Campana et al., 2002; Goldman et al., 2006; Natanson et al., 2006, 2008; Piercy et al., 2007; Semba et al., 2009). The complexity of growth band deposition rate in sharks is evidenced by a recent study demonstrating that although annual deposition has been validated in adult *I. oxyrinchus*, juveniles from southern California have a biannual deposition rate (Wells et al., 2013).

In the present study, the difference of growth band counts along the vertebral column supports somatic growth band deposition, with large vertebrae having more growth bands. Such variation has also been observed in other species for which somatic growth band deposition has been demonstrated, such as the basking sharks (Natanson et al., 2008) or the Pacific angel shark (Natanson and Cailliet, 1990). The discrepancy in growth band count along the vertebral column has, however, also been shown in species with seasonal growth band deposition (Officer et al., 1996), and might be due to the difficulties associated with growth rings being more concentrated and harder to count on small vertebrae.

Wobbegongs with known length-at-age and captive growth rates fitted better to growth models when it was assumed that sharks deposited two growth bands per year than when assuming annual growth band deposition. At the same time, a note of caution must be applied to this result as for some species, captive growth can be as much as nine or ten times faster than natural growth (e.g. *Negaprion brevirostris* – Gruber and Stout, 1983; and *Carcharhinus amblyrhynchus* – Waas, 1971). Therefore, if accelerated growth in captive wobbegongs occurred, the captive growth rate would have had a better fit with growth models when it was assumed that sharks deposited one growth band per year, similar to other members of their taxa.

In this study, growth band deposition was more likely linked to somatic growth than seasonality. However, the likely overestimated captive growth rate (Gruber and Stout, 1983; Waas, 1971), the average growth band formation rate obtained from fluorochrome labelling, and findings from previous studies on Orectolobidae (Chidlow et al., 2007; Tanaka, 1990) suggest that the

average growth bands formation is approximately annual. Assuming that growth band formation is on average annual, and using the model with the best fit to the data and taking into account biologically meaningful estimations of L_{∞} and k , growth parameters obtained were: 999, 1630 and 2128 mm TL for L_{∞} and 0.19, 0.09 and 0.20 for k for *O. ornatus*, *O. maculatus*, and *O. halei*, respectively. Growth parameters estimated for *O. halei* in WA (misidentified as *O. ornatus*) using counts obtained from X-rays applying the VBGM on observed lengths-at-age combined with back-calculated lengths (using Dahl–Lea direct proportion method) were 0.099 for k , and 2229 mm for L_{∞} (Chidlow, 2003). The present study obtained similar estimates ($k = 0.09$ and $L_{\infty} = 2401$ mm) when using the VBGM. However, the maximum age of individuals sampled was less in the present study (26 increments) compared to individuals from WA (32 increments).

The inconsistent results from the age validation (fluorochrome labelling) studies suggest that longer term inter-period labelling or other techniques need to be used to determine the periodicity, or otherwise, of growth band deposition. The bomb radiocarbon method (Campana et al., 2002; Druffel and Linick, 1978) as a method for age validation is not applicable for wobbegongs because samples during the C^{14} increase (1955–1970) are not available. Electron microprobe analysis of calcium and phosphorus weight-fraction concentration might provide some information on the periodicity of band deposition (Cailliet and Radtke, 1987). Simple demographic analysis combining estimated fecundity, age-at-maturity and longevity may also help determine which growth band deposition rate is more biologically sound by comparing reproductive output.

The present study is the first to use wild chemically marked Orectolobiformes to attempt to validate growth band deposition rate. Our results support previous findings that growth band deposition in orectolobids are more likely to be related to somatic growth than seasonality. According to the average growth band formation obtained from chemical marking of wild and captive individuals, and accounting for potentially overestimated captive growth, the periodicity of growth band deposition approximated an annual pattern, which was used to produce age and growth parameters for three sympatric wobbegong species. These are the first growth parameter estimates for these commercially caught species, and while it is important to be cautious in their application, they provide information essential for stock assessment and for demographic analyses needed to ensure the sustainability of the harvest of these species. As previously recommended by Chidlow et al. (2007), further work is, however, needed to better understand the factors underlying the formation of growth bands in *Orectolobus* spp.

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