ISC/14/SHARKWG-3/05

Evaluation of growth band counts precision in the vertebrae of shortfin mako sharks caught in the Mexican Pacific[1]

Prepared by

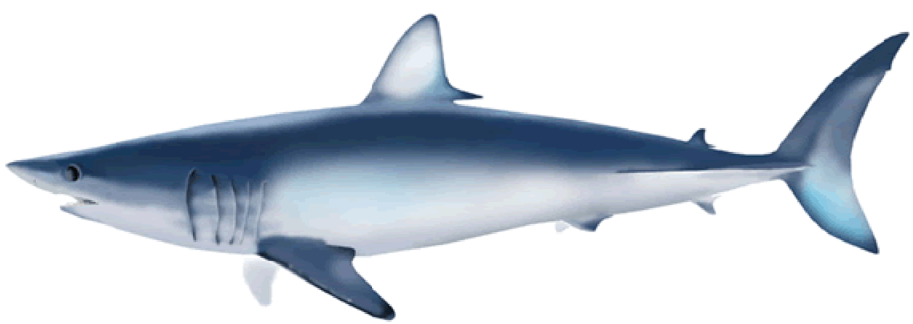
Instituto Nacional de Pesca:

Javier Tovar-Ávila, Darío A. Chávez-Arrenquín, J. Leonardo-Castillo-Géniz, David Corro-Espinosa, Carlos J. Godínez-Padilla, Gustavo Andrade-Domínguez and Amado Torres

Universidad Autónoma de Sinaloa:

J. Fernando Márquez-Farías, Raúl E. Lara-Mendoza, Jesús E. Osuna Soto, Allan Rosales Valencia and Luis Daniel Carrillo Collín

Email address of contact
javiertovar.mx@gmail.com



[1]Working document submitted to the ISC Shark Working Group Workshop, 19-26 November 2014, Puerto Vallarta, Jalisco, Mexico. **Document not to be cited without author's permission.**

INTRODUCTION

The age of the shortfin mako shark, *Isurus oxyrinchus*, from Mexican waters has been estimated previously by counting growth bands in whole vertebrae stained with silver nitrate, from 109 individuals caught during 2000–2003 off the western coast of Baja California Sur. The precision of growth band counts in this study was estimated by calculating the coefficient of variation (CV) (8.9%) of three different readers. Estimated ages in this study ranged 0–18 years, with the majority of fish being 1–5 years old, based in the results of vertebral edges analysis which suggested the annual formation of growth band pairs. The authors stated in this study, however, the necessity to corroborate the periodicity of growth bands during all lifespan (Ribot-Carballal *et al*. 2005).

The age of *I. oxyrinchus* has also been determined in the western and central North Pacific Ocean, by counting growth bands on half-cut vertebral centra of 275 individuals using a shadowing method. In this method the ridges on the surface of the centra (consisting of a convex and concave structure) were counted. The precision of counts was also estimated with the CV (3.7%) and similarly to the study from Mexican waters, the centrum edge analysis suggested an annual formation of growth band pairs (Semba *et al*. 2009).

Direct validation studies (using OTC and X-radiographies of sectioned vertebrae) have proven recently, however, that at least for the first five years of age, two band pairs (two translucent and two opaque) are formed each year for the species in southern California (Wells *et al*. 2013).

Given the high degree of uncertainty on age estimations of *I. oxyrinchus* and other pelagic sharks, the Shark Working Group (SHARKWG) of the International Scientific Committee for Tuna and Tuna-like Species in the North Pacific Ocean (ISC), has highlighted the need to take action to standardize protocols for sampling collections, processing and data analysis. The SHARKWG-ISC has recommended conducting cross-validation studies that may be useful to derive a unified growth curve for shortfin mako in the North Pacific based on the vertebrae shared among laboratories (SHARKWG-ISC 2014). In the present document we report the results of an inter-research group crossed experiment of growth band counts, using vertebrae of *I. oxyrinchus* caught in Mexican waters. The aim of such comparison was to determine the convenience of a standard, non-expensive and time consuming methodology that can ensure precise age estimates, in terms of repeatability of growth band counts. Precision estimates of vertebrae samples shared by other members of the SHARKWG-ISC, processed with the same methodology than the vertebrae from Mexican waters sharks, are presented as well in a preliminary way (Annex 1).

MATERIAL AND METHODS

Vertebrae collection and preparation

Isurus oxyrinchus vertebrae were collected from landings of the artisanal and industrial fleets operating along the North Pacific Mexican coast from 2007 to 2014. Due to de the difficulty to obtain vertebrae located below the first dorsal fin, particularly in the artisanal fishery, some samples were obtained from the cervical region once the shark was beheaded. The samples were preserved frozen until their preparation.

The vertebrae were processed following a similar methodology by two independent research groups, the shark research group at the Faculty of Marine Sciences from the Autonomous University of Sinaloa (FACIMAR-UAS) and the shark group of the National Fisheries Institute (INAPESCA) (the counts corresponding to each research group are not identified purposely).

Each vertebra was mounted on a piece of wood and sagitally sectioned through the focus with a slow speed saw (Buehler, Model Isomet), using a single or twin blades with diamond edge (4 x 0.001" Buehler 11-4244) to obtain sections with a thickness of 0.4–0.35 mm. All vertebrae sections were photographed with a digital camera (OptixCam OCS-10 MP) adapted to a stereomicroscope with transmitted light (Olympus SZ61) and the photographs were analyzed using Image Pro Plus 7.0 imaging software.

Vertebrae from different regions of the Pacific Ocean, shared by other members of the SHARKWG-ISC, were processed with the same methodology than the vertebrae of sharks from Mexican waters. Though some of these vertebrae were received already cleaned, it was assumed the cleaning process was similar having no influence in the section and observation process.

Growth bands identification and counts

The protocol to determine and count growth bands involved as first step the identification of the birthmark, which was determined as the fully formed band pair (or the first hyaline growth band) beyond the vertebral focus that was associated with the angle change in the *corpus calcareum* (*CC*). A pre-birth mark (also associated to an angle change in the CC) was located near the origin of several vertebrae, but was not considered in growth counts. Further standardization of the criteria to identify and count the growth band counts was discussed at the internal of each research group, but not between both groups deliberately.

A legibility score of the quality of growth bands counts was established (1 was low and 5 was the best quality) and the edge type (opaque and hyaline) recorded (Officer *et al.* 1996).

Counts of the growth bands were performed in the photographs obtained. To estimate the repeatability of counts, each vertebra was read independently by two readers of each research group. If the number of counts differed between readers a third count was undertaken. In every case, two readers reach an agreement for the final number of growth bands in order to get the inter research group comparison. The frequency of final growth band counts agreed by each research group were plotted, but they were not related to age estimations due to the inconsistencies in age validation to date.

Estimation of precision and bias

The average percentage error (APE) (Beamish and Fournier 1981) and CV (Chang 1982) were used to estimate the precision of growth bands counts between readers of each research group and among them using the final growth band number agreed by each group:

$$APE = 100\left\{\frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{\left|X_{ij}-X_{j}\right|}{X_{j}}\right]\right\}$$

$$CV = 100 * \frac{\sqrt{\sum_{i=1}^{R}\frac{\left(x_{ij}-x_{j}\right)^{2}}{R-1}}}{x_{j}}$$

Where N is the number of vertebrae; R is the number of readings of individual j; X_{ij} is age i determined for individual j; and X_j is the mean age calculated for individual j.

Age-bias plots (Campana 2001) were also constructed to identify consistency between readers and research groups, as well as possible systematic bias. Consistency tables and the Bowker test of Symmetry were also performed by the FACIMAR-UAS research group in order to compare intra group counts precision for different sets of vertebrae (Annex 1).

RESULTS AND DISCUSSION

A total of 66 vertebrae from sharks caught in Mexican waters were processed and analyzed, of which 47 were collected by INAPESCA and 19 by FACIMAR-UAS. Vertebrae from 58 sharks provided by SHARKWG-ISC were also processed.

All vertebrae showed visible growth bands, including some vertebrae which presented only the birthmark and prenatal marks (Fig. 1). Such prenatal mark was reported previously by Ribot-Carballal *et al.* (2005), who suggested its probable relation to an embryonic change in nutrition associated with the peculiar reproductive mode of lamnoid sharks, as similar marks have been reported for the sandtiger shark, *Carcharias taurus* (Branstetter and Musick 1994 in Ribot-Carballal *et al*. 2005).

Both research groups identified the same amount of hyaline and opaque edges, being the opaque the most common 98.6%. This indicated that the method used allowed for a good degree of consistency in the identification of the vertebral edge type.

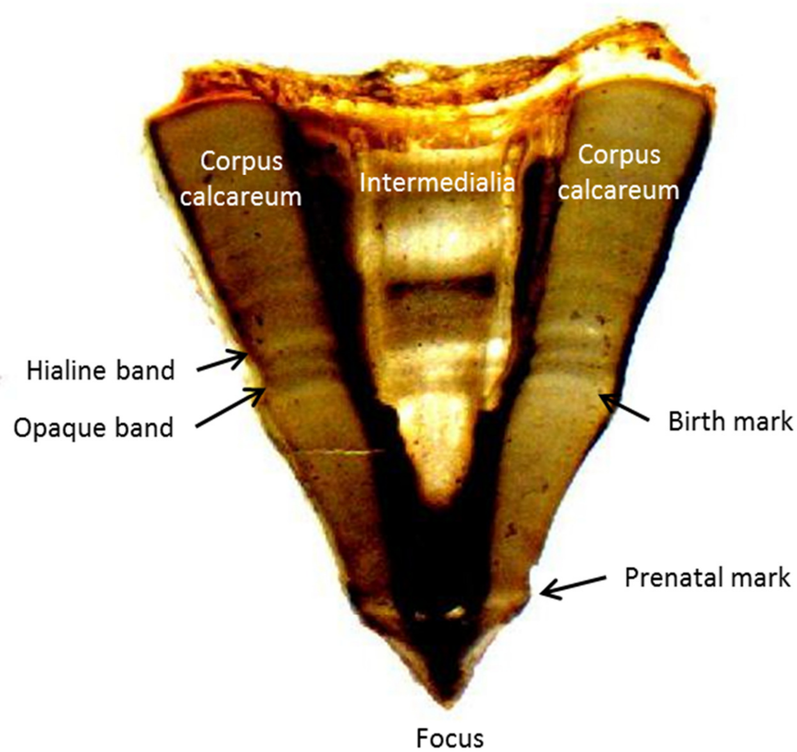


Fig. 1. Sagittal section of *Isurus oxyrinchus* vertebrae, observed with transmitted light and photographed with a digital camera (OptixCam OCS-10 MP) and Image Pro Plus 7.0 imaging software adapted to a stereomicroscope (Olympus SZ61). 1.0x.

The average readability of the vertebrae estimated by the first research group was 3.9 (Std. Dev. 1.6), similar to the second research group score for all vertebrae, with an average of 3.4 (Std. Dev. 0.9).

The number of growth bands observed in the vertebrae varied from 0 to 16 for the research group 1 (average= 7.09), being the most common the group of vertebrae with seven growth bands, whereas for the research group 2 the number of growth bands varied from 0 to 15 (average= 7.34) being the most common the group of vertebrae with five and eight growth bands (Fig. 2).

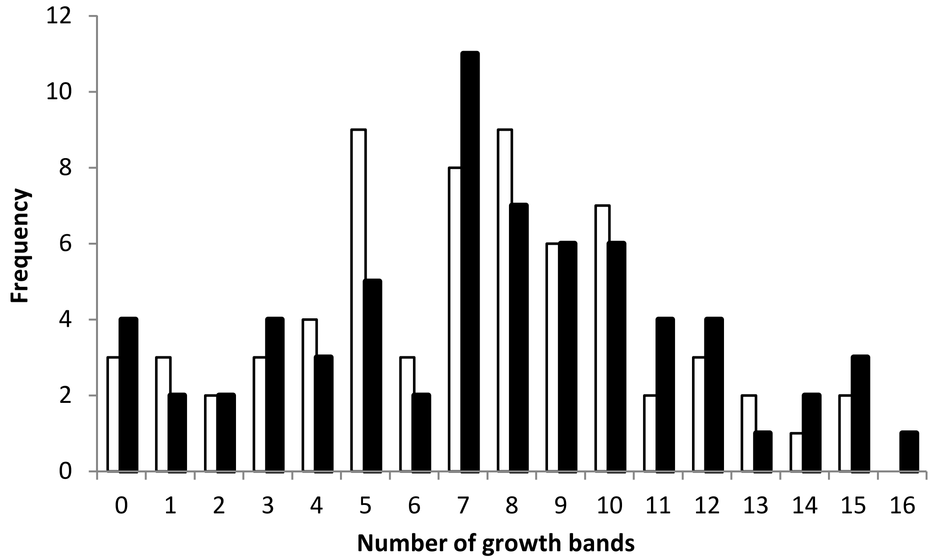


Fig. 2. Frequency distribution of the number of growth band counted by research group 1 (dark bars) and research group 2 (white bars).

The APE and CV for all vertebrae estimated by the first research group was 4.03% and 5.79% respectively, whereas the second research group estimated a higher error with an APE of 5.7% and CV of 5.4%. The APE and CV estimated between the final counts of both research groups was 5.85% and 8.14% respectively.

The age bias plots showed a high consistency of growth band counts with no systematic bias for counts of each research group (Fig. 3). However, a slight systematic bias was detected in the comparison between the research groups counts (Fig. 4). Similar biases have been detected in other studies comparing growth band counts performed by several age specialized laboratories (e.g. the spiny dogfish, *Squalus ancanthias*) (Rice *et al.* 2009). Such results proved that ageing sharks is a highly subjective process that needs a further inter-laboratory standardization of criteria to identify growth bands, despite the structure produce apparent and easy to identify growth bands.

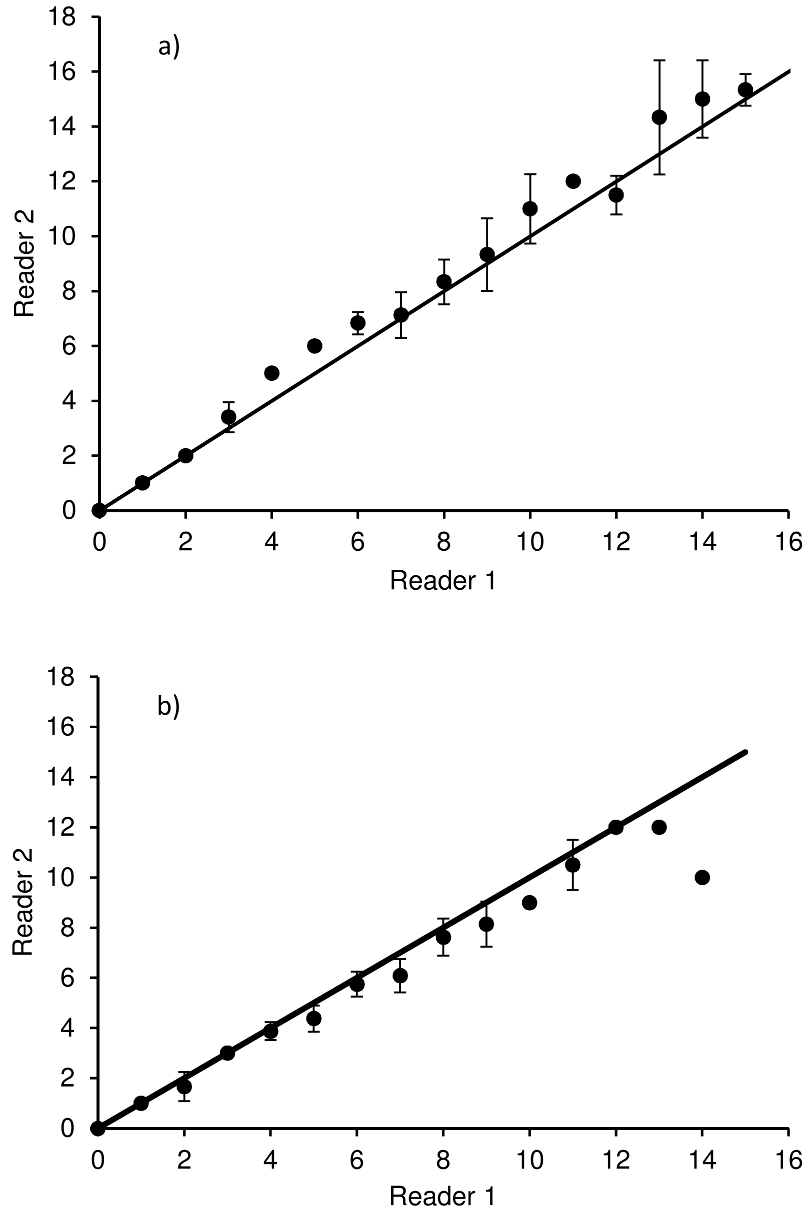


Fig. 3. Age bias plots for growth band counts of a) research group 1 and b) research group 2.

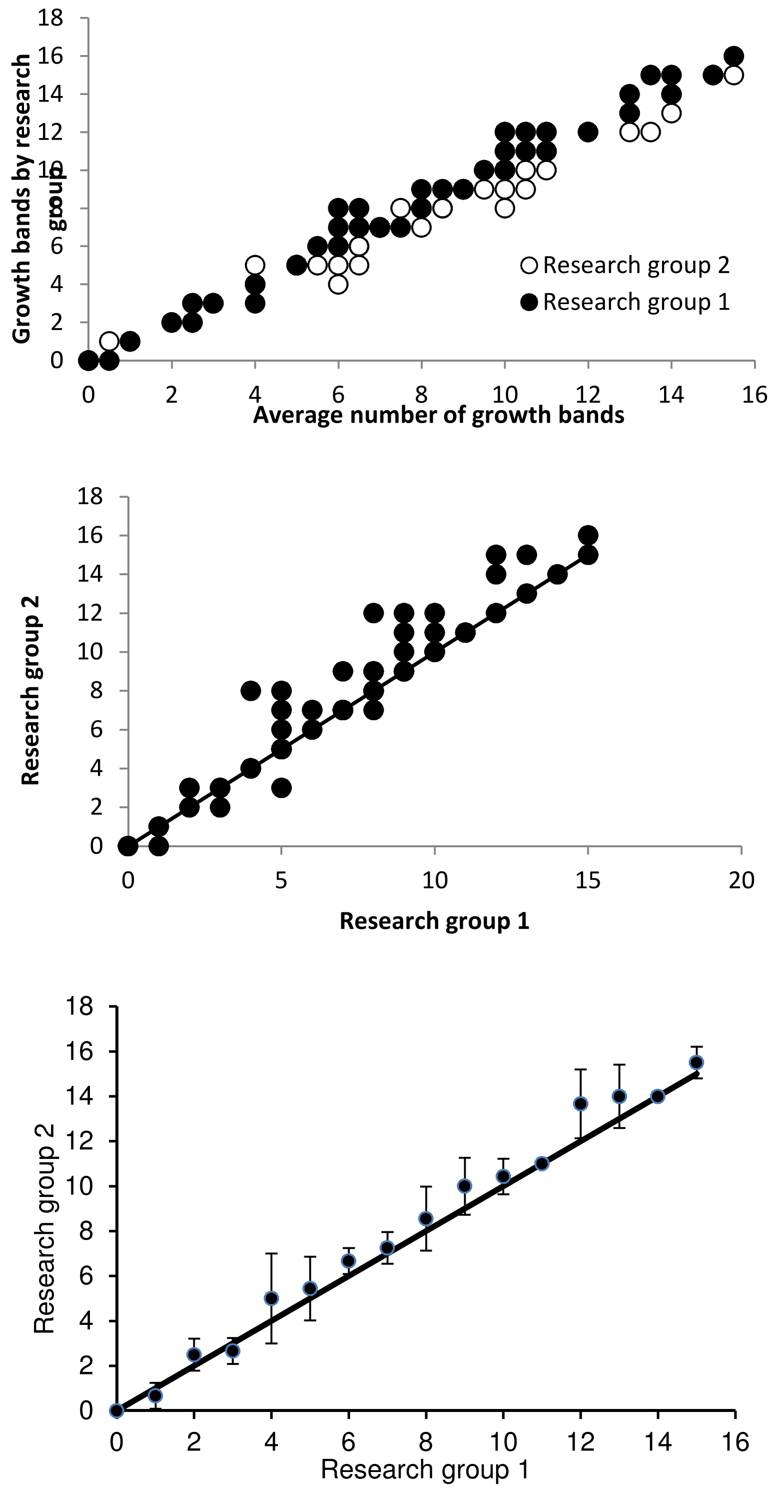


Fig. 4. Age bias plots comparing growth band counts of both research groups.

CONCLUSIONS

- Sagittal sections of shortfin mako sharks (*Isurus oxyrinhus*) vertebrae, observed under a stereoscopic microscope with transmitted light, photographed and processed with the Image Proplus program provide a highly reproducible method to count growth band marks. Such method is relatively cheap and low time consuming, allowing the interchange of information and samples among research groups.

- The standardization of growth bands criteria previous to undertake counts is necessary to achieve comparable and not bias estimations between different readers and research groups. Further analysis is needed to determine if the systematic biases detected between the research groups produce significant differences in the estimation of growth parameters for *I. oxyrinchus*.

- Direct validation of the growth band formation of sharks of all ages and from different regions of the Pacific Ocean is urgently needed, to determine the relationship between growth band number estimated in the vertebrae of a shark and its age.

REFERENCES

Beamish R.J. & Fournier D.A. 1981. A method for comparing the precision of a set of age determinations. *Can J Fish Aquat Sci* 38: 982–983.

Campana S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J Fish Biol* 59: 197–242.

Chang W.Y.B. 1982. A statistical method for evaluating the reproducibility of age determination. *Can J Fish Aquat Sci* 39: 1208–1210.

Officer R.A., Gason A.S., Walker T. I. ans Clement J. G. 1996. Sources of variation in counts of growth increments in vertebrae from gummy shark, (*Mustelus antarcticus*, and school shark, *Galeorhinus galeus*): implications for age determination. *Can J Fish Aquatic Sci* 53(8): 1765-1777.

Ribot-Carballal M.C., Galván-Magaña F. & Quiñónez-Velázque C. 2005. Age and growth of the shortfin mako shark, *Isurus oxyrinchus*, from the western coast of Baja California Sur, Mexico. *Fish Res* 76: 14–21.

Rice J.S., Galluci V. F & Kruse G. H. 2009. Evaluation of the precision of age estimates for spini dogfish. Pp. 161-168. In: Galluci et al. (eds) Biology and management of dogfish sharks. American Fisheries Society. U.S.

Semba Y., Nakano H. & Aoki I. 2009. Age and growth analysis of the shortfin mako, *Isurus oxyrinchus*, in the western and central North Pacific Ocean. *Environ Biol Fish* 84:377–391.

Wells R.J.D., Smith S.E., Kohin S., Freund E., Spear N. & Ramon D.A. 2013. Age validation of juvenile Shortfi n Mako (*Isurus oxyrinchus*) tagged and marked with oxytetracycline off southern California. *Fish Bull* 111:147–160.

ANNEX 1

The intra-reader comparison undertaken by the FACIMAR-UAS research group for each set of vertebrae analyzed is presented in the present annex.

The Bowker test of Symmetry did not reveal significant differences between readers for the FACIMAR-UAS (X_i^2= 16.8, p > 0.27) (Table 1), INAPESCA (X_i^2= 17.8, p > 0.27) (Table 2) and SHARKWG-ISC vertebrae sets (X_i^2= 24, p > 0.34) (Table 3).

Table 1. Contingency table comparing the number of bands between readers for the UAS vertebrae set.

		Reader 1																	
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**Total**
Reader 2	**0**	3																	3
	1		1																1
	2																		0
	3				1														1
	4				1														1
	5																		0
	6							1											1
	7								2										2
	8									1	1								2
	9																		0
	10									1		2							3
	11										1								1
	12											1	1						2
	13																		0
	14																		0
	15																		0
	16														1	1			2
	Total	**3**	**1**	**0**	**2**	**0**	**0**	**1**	**2**	**2**	**2**	**3**	**1**	**0**	**1**	**1**	**0**	**0**	**19**

Table 2. Contingency table comparing the number of bands between readers for the INAPESCA vertebrae set.

		Reader 1																	
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**Total**
Reader 2	**0**	1																	1
	1		1																1
	2			2															2
	3				2														2
	4				1														1
	5					5													5
	6						1		1										2
	7							4	5										9
	8									4	1								5
	9								1	1	4								6
	10										1								1
	11											1		1					2
	12										1		1	1	1				4
	13												1						1
	14														1				1
	15														1		2		3
	16																1		1
	Total	**1**	**1**	**2**	**3**	**5**	**1**	**4**	**7**	**5**	**7**	**1**	**2**	**2**	**3**		3		**47**

Table 3. Contingency table comparing the number of bands between readers for the ISCMAK vertebrae set.

		Reader 1																										
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**Total**
Reader 2	**0**	6																										6
	1		3																									3
	2			2																								2
	3				2																							2
	4					1																						1
	5																											0
	6																											0
	7								2		1																	3
	8								1	1	2																	4
	9										2																	2
	10											1																1
	11												3															3
	12														2													2
	13															1	1	1										3
	14																											0
	15														1		2											3
	16														1		2	1										4
	17															1		1				1						3
	18																	3			1							4
	19																	1		1								2
	20																			1		1			1			3
	21																			1	1	1						3
	22																									1	1	2
	23																									1		1
	24																									1		1
	25																											0
	Total	**6**	**3**	**2**	**2**	**1**	**0**	**0**	**3**	**1**	**5**	**1**	**3**	**0**	**4**	**2**	**5**	**7**	**0**	**3**	**2**	**3**	**0**	**0**	**1**	**3**	**1**	**58**

Similarly, the age bias plots indicated that both readers identified a similar number of bands in each set of vertebrae: FACIMAR-UAS (Fig. 5), INAPESCA (Fig. 6) and SHARKWG-ISC (Fig. 7).

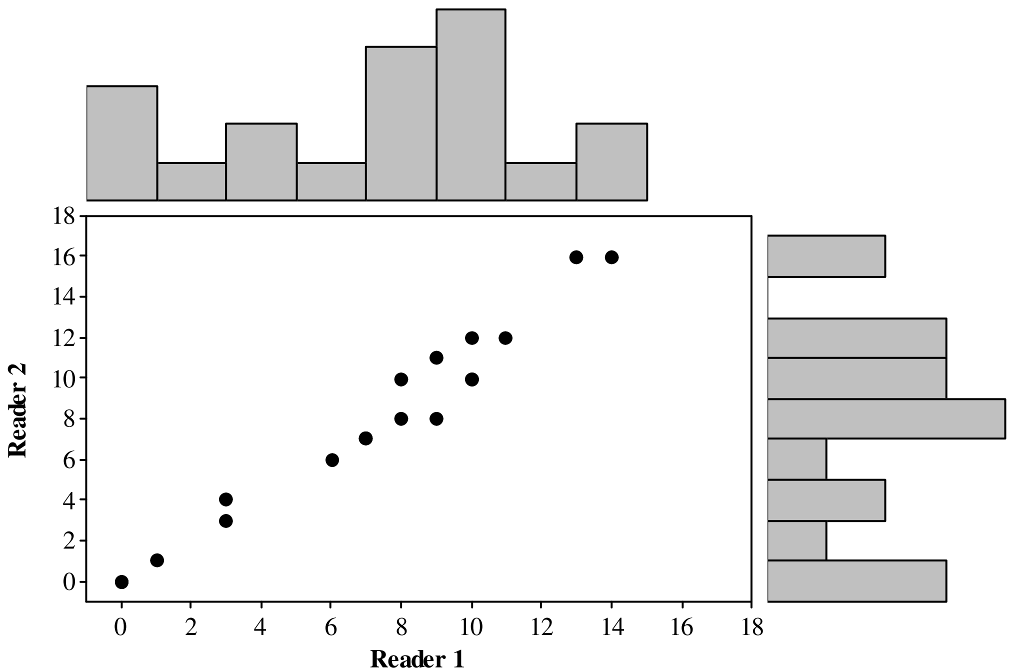


Fig. 5. Relationship between two readers and frequency distribution of the number of bands for the UAS vertebrae set. The corresponding frequency distribution of number of bands is presented.

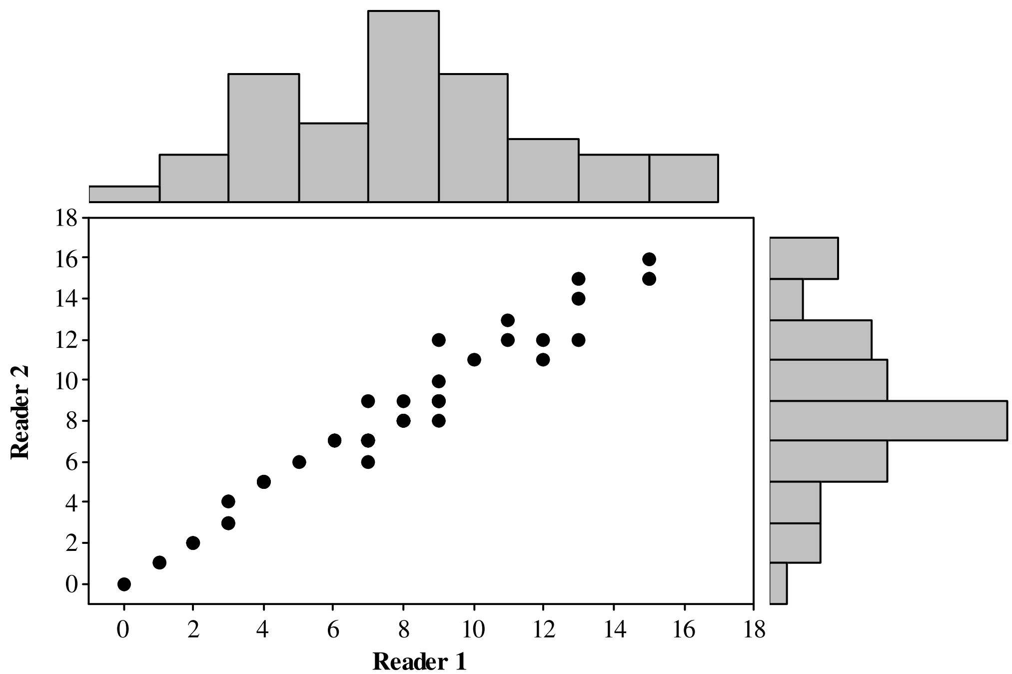


Fig. 6. Relationship between two readers and frequency distribution of the number of bands for the INAPESCA vertebrae set. The corresponding frequency distribution of number of bands is presented.

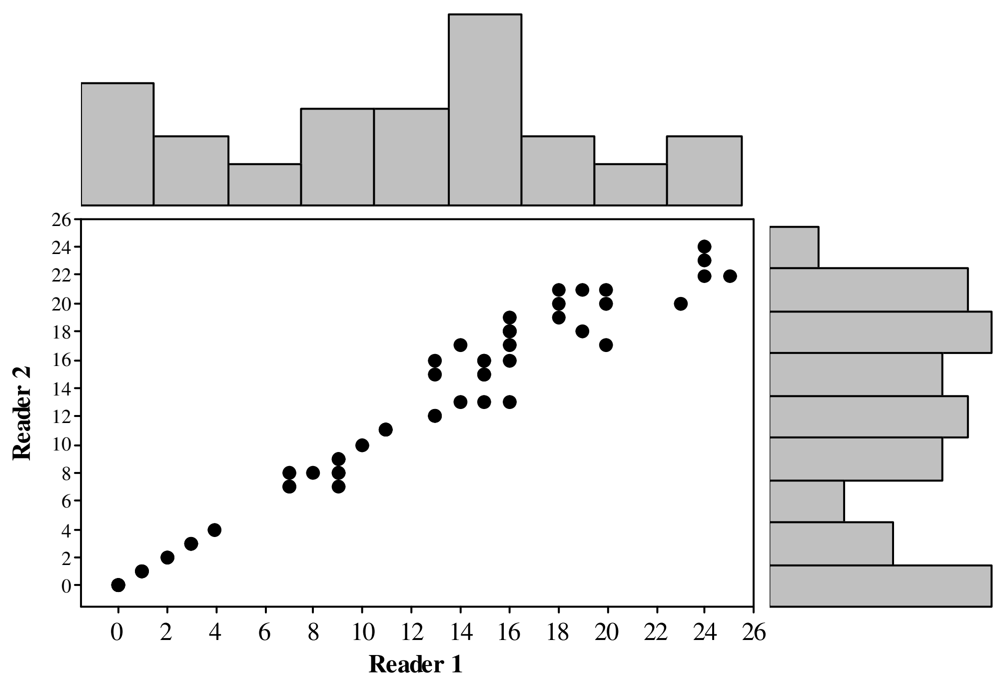


Fig. 7. Relationship between two readers and frequency distribution of the number of bands for the ISCMAK vertebrae set. The corresponding frequency distribution of number of bands is presented.

The APE of the different vertebrae sets ranged between 3.05 and 4.23, whereas the *CV* ranged between 4.31 and 5.98, presenting the SHARKWG-ISC vertebrae the lowest and the INAPESCA vertebrae the highest error (Table 4). However, growth band counts from all the sets of vertebrae were considered with a high degree of precision and repeatability.

Table 4. Number of vertebrae, average percentage error and variation coefficient for each set vertebrae (ISC, INAPESCA and UAS).

	n	APE	CV
ISC	58	3.05	4.31
INAPESCA	47	4.23	5.98
UAS	19	3.78	5.34