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Counting of concentric bands in vertebrae of shortfin mako shark of the North Pacific Ocean[1]

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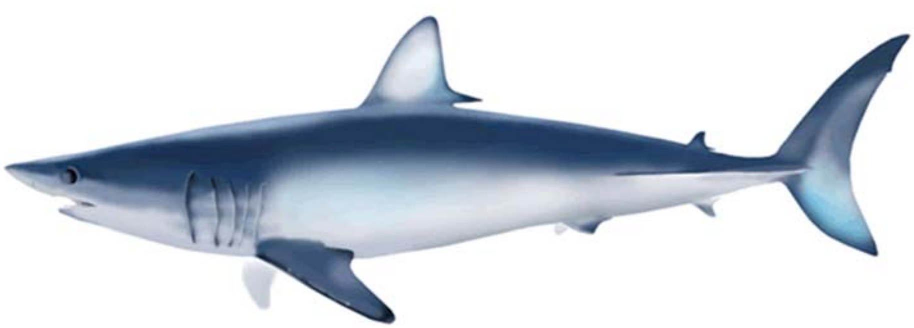

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1. INTRODUCTION

Given the high degree of uncertainty on key parameters associated with age and growth of shortfin mako 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), have highlighted the need to take action to standardize protocols for sampling collections, processing and data analysis. The ISC sponsored a Shark age and growth Workshop, held in November 2011 in La Jolla, California, with the aim to bring together specialists from ISC member nations to discuss methodologies and regional studies on age and growth of shortfin mako and blue sharks and develop collaborative plans. In a second meeting held January 2014 in La Jolla, California, the SHARKWG meet again with the objectives to review recent developments on age and growth of shortfin mako. One of the main tasks that resulted from the second meeting was to conduct 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 counting of growth bands in vertebrae of mako shark observed in the digital image shared by members of the SharkWG-ISC.

2. MATERIAL AND METHODS

2.1 Age structure preparation

The vertebrae of shortfin mako shark were analyzed at Facultad de Ciencias del Mar of Universidad Autónoma de Sinaloa (FACIMAR-UAS). Each vertebra was mounted on a piece of wood with its respective code and was sagitally cut through the focus using a slow speed saw (Buehler, Model Isomet 111280-160) with a thickness of 0.4 mm, using twin blades with diamond edge (4 x 0.001" Buehler 11-4244). Cut 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.

2.2 Growth bands readings

We used the photographs to count the number of growth bands in the vertebrae of shortfin mako. In order to estimate the repeatability of the counting, each vertebra was read by two readers. If the number of counts differed between readers a third count was done. In every case, two readers reach an agreement for the final number of growth bands. The protocol we use was as follows. First, we identify the birthmark (age- 0), which was determined as the fully formed band pair beyond the focus that was associated with the angle change in the *corpus calcareum* (*CC*). Then, we count the opaque and hyaline bands on the *CC*, the edge type (opaque or hyaline) and a score to note the quality and concordance between readings (1 was low and 5 was the best quality and agreement).

2.3 Reader precision/bias

The average percentage error (APE) (Beamish and Fournier, 1981) and coefficient of variation method (CV) (Chang, 1982) was calculated to compare the reliability of the readings, according to equation 1 and 2, respectively:

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

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

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*.

An age-bias plot (Campana, 2001) was used to identify trend and consistency between readers. Then, a contingency table and corresponding plots of comparing reader one's and reader two's ages was build to visually assess differences between readers. All the material utilized and detal of the counting are presented in Apendix I.

3. RESULTS

3.1 Verification and precision analysis

3.1.1 Bowker´s Test of Symmetry

The comparison of number of bands counted by readers shows an acceptable pattern of symmetry. Bowker test did not reveal significant differences between readers for the UAS (Universidad Autónoma de Sinaloa) (X_i^2= 16.8, p > 0.27) (Table 1), INAPESCA (X_i^2= 17.8, p > 0.27) (Table 2) and ISCMAK vertebrae set (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**

3.1.2 Age-bias plot

The comparison of counts between readers indicated that two readers were identified the same number of bands in the vertebrae. The relationship of the number of bands between readers and the corresponding frequency distribution of number of bands is presented for each Lab; UAS (Fig. 1), INAPESCA (Fig. 2) and ISCMAK (Fig. 3).

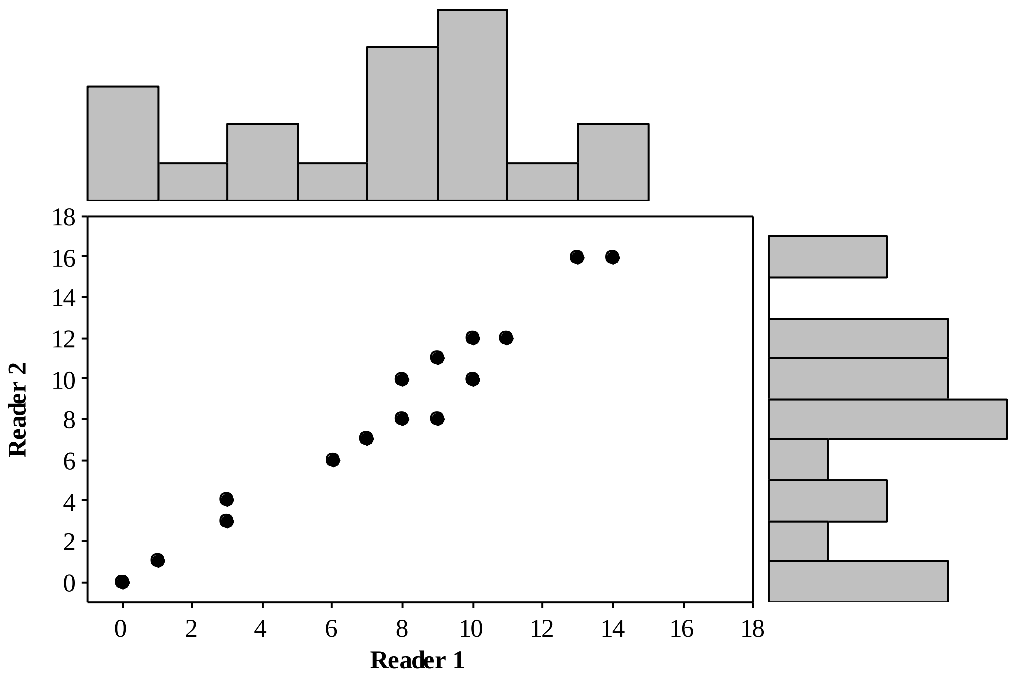

Fig. 1. Relationship between two readers and frequency distribution of the number of bands for the UAS vertebrae set.

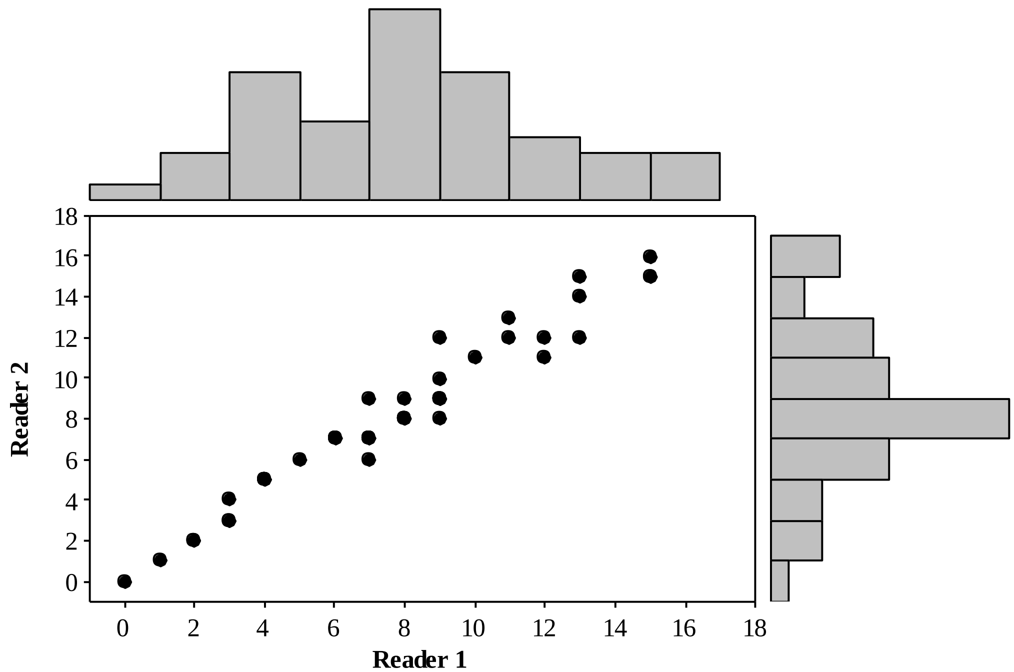

Fig. 2. Relationship between two readers and frequency distribution of the number of bands for the INAPESCA vertebrae set.

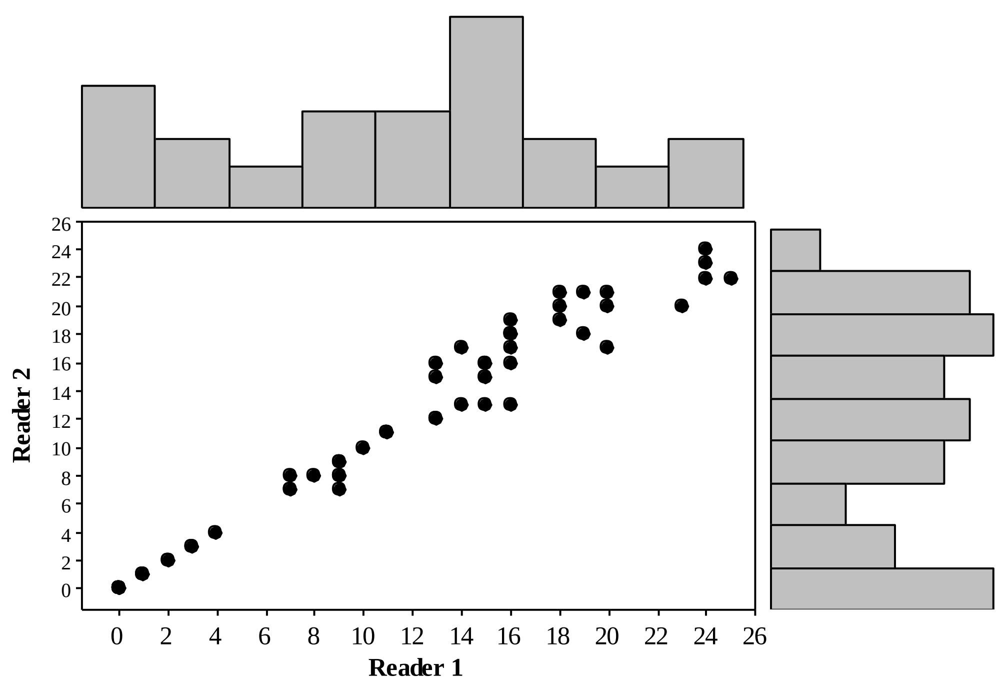

Fig. 3. Relationship between two readers and frequency distribution of the number of bands for the ISCMAK vertebrae set.

3.1.3 Average percentage error (APE) and coefficient of variation (CV)

The average percentage error ranged between 3.05 and 4.23, where ISC vertebrae were lowest and INAPESCA vertebrae the highest. Coefficient of variation (*CV*) oscillated between 4.31 and 5.98 and were considered a low value by age determination (Table 4).

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

4. REFERENCIAS

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.

Apendix I

Material: Universidad Autónoma de Sinaloa-Mexico

Code	Reader 1	Reader 2	Agreement	Score	Edge
ISO_1_UAS	3	4	3	3	Hialine
ISO_2_UAS	10	10	10	4	Opaque
ISO_3_UAS	8	8	8	4	Hialine
ISO_4_UAS	6	6	6	4	Opaque
ISO_5_UAS	10	10	10	3	Opaque
ISO_6_UAS	10	12	10	2	Opaque
ISO_7_UAS	13	16	13	1	Opaque
ISO_8_UAS	11	12	12	2	Opaque
ISO_9_UAS	1	1	1	5	Opaque
ISO_10_UAS	7	7	7	4	Opaque
ISO_11_UAS	9	8	8	2	Opaque
ISO_12_UAS	7	7	7	3	Opaque
ISO_13_UAS	9	11	11	2	Hialine
ISO_14_UAS	14	16	14	1	Opaque
ISO_15_UAS	3	3	3	5	Opaque
ISO_16_UAS	0	0	0	5	Opaque
ISO_17_UAS	8	10	10	3	Opaque
ISO_18_UAS	0	0	0	5	Hialine
ISO_19_UAS	0	0	0	5	Opaque

***Apendix I* (cont…)**

Material: INAPESCA-Mexico

Code	Reader 1	Reader 2	Agreement	Score	Edge
ISO_1_INAPESCA	4	5	5	3	OPAQUE
ISO_2_INAPESCA	6	7	7	2	OPAQUE
ISO_3_INAPESCA	3	4	4	2	OPAQUE
ISO_4_INAPESCA	2	2	2	4	OPAQUE
ISO_5_INAPESCA	7	9	9	3	OPAQUE
ISO_6_INAPESCA	7	7	7	3	OPAQUE
ISO_7_INAPESCA	9	9	9	3	OPAQUE
ISO_8_INAPESCA	6	7	7	4	OPAQUE
ISO_9_INAPESCA	8	8	8	3	OPAQUE
ISO_10_INAPESCA	9	9	9	3	OPAQUE
ISO_11_INAPESCA	0	0	0	5	HYALINE
ISO_12_INAPESCA	4	5	4	3	OPAQUE
ISO_13_INAPESCA	4	5	5	3	HYALINE
ISO_14_INAPESCA	4	5	4	3	OPAQUE
ISO_15_INAPESCA	4	5	5	3	OPAQUE
ISO_16_INAPESCA	5	6	5	3	OPAQUE
ISO_17_INAPESCA	7	6	6	3	OPAQUE
ISO_18_INAPESCA	7	7	7	3	OPAQUE
ISO_19_INAPESCA	9	12	12	3	OPAQUE
ISO_20_INAPESCA	11	12	11	3	OPAQUE
ISO_21_INAPESCA	13	15	15	2	OPAQUE
ISO_22_INAPESCA	10	13	10	3	OPAQUE
ISO_23_INAPESCA	9	9	9	3	OPAQUE
ISO_24_INAPESCA	6	7	7	3	OPAQUE
ISO_25_INAPESCA	3	3	3	4	OPAQUE
ISO_26_INAPESCA	14	14	14	4	OPAQUE
ISO_27_INAPESCA	9	9	9	5	OPAQUE
ISO_28_INAPESCA	10	10	10	4	OPAQUE
ISO_29_INAPESCA	12	12	12	4	OPAQUE
ISO_30_INAPESCA	13	12	12	4	OPAQUE
ISO_31_INAPESCA	6	7	7	4	OPAQUE
ISO_32_INAPESCA	7	7	7	5	HYALINE
ISO_33_INAPESCA	15	16	16	4	OPAQUE
ISO_34_INAPESCA	15	15	15	3	HYALINE
ISO_35_INAPESCA	9	8	8	3	OPAQUE
ISO_36_INAPESCA	7	7	7	3	OPAQUE
ISO_37_INAPESCA	3	3	3	5	OPAQUE
ISO_38_INAPESCA	2	2	2	4	OPAQUE
ISO_39_INAPESCA	1	1	1	4	OPAQUE
ISO_40_INAPESCA	8	8	8	5	OPAQUE
ISO_41_INAPESCA	8	8	8	4	OPAQUE
ISO_42_INAPESCA	8	8	8	4	HYALINE
ISO_43_INAPESCA	9	9	9	4	OPAQUE
ISO_44_INAPESCA	15	15	15	4	OPAQUE
ISO_45_INAPESCA	12	11	11	3	OPAQUE
ISO_46_INAPESCA	10	11	11	3	OPAQUE
ISO_47_INAPESCA	6	7	7	3	OPAQUE

***Apendix I* (cont…)**

Material: ISC

Code	Reader 1	Reader 2	Agreement	Score	Edge
ISCMAK-01_1X_1	13	12	12	3	OPACO
ISCMAK-10_1X	16	17	17	2	OPACO
ISCMAK-19_1.2X	24	22	22	2	OPACO
ISCMAK-21_1X	19	21	21	2	OPACO
ISCMAK-22_1X_2	15	16	16	3	OPACO
ISCMAK-26_1X	15	16	16	3	OPACO
ISCMAK-30_1X	16	18	16	2	HIALINO
ISCMAK-33_1X	18	19	19	4	OPACO
ISCMAK-35_1X	24	23	23	4	OPACO
ISCMAK-37_1X_2	20	21	20	3	HIALINO
ISCMAK-40_1.2X_	11	11	11	5	OPACO
ISCMAK-40_1X_1	15	15	15	4	OPACO
ISCMAK-41_1X_1	13	15	15	3	OPACO
ISCMAK-44_1X	24	24	24	3	OPACO
ISCMAK-53_1X_2	13	12	13	4	OPACO
ISCMAK-56_1X	13	16	16	2	HIALINO
ISCMAK-58_1X	18	20	18	2	OPACO
ISCMAK-59_1X	16	19	19	3	OPACO
ISCMAK-61_1X_1	25	22	22	2	OPACO
ISCMAK-62_1X	15	15	15	3	OPACO
ISCMAK-65_1X_2	20	20	20	3	OPACO
ISCMAK_06_TWN_1.0X	23	20	20	2	OPACO
ISCMAK_07_TWN_3x2	0	0	0	5	OPACO
ISCMAK_13_TWN_1.2X	11	11	11	4	OPACO
ISCMAK_16_TWN_1.2X1	16	18	16	2	OPACO
ISCMAK_17_TWN_1.2X	11	11	11	3	OPACO
ISCMAK_18_TWN_1.2X_2	16	13	13	3	HIALINO
ISCMAK_20_TWN_1.2X_1	16	18	18	1	OPACO
ISCMAK_24_TWN_1.0X2	20	17	17	1	OPACO
ISCMAK_29_TWN_1.2x	10	10	10	2	OPACO
ISCMAK_31_TWN_3x1	2	2	2	3	HIALINO
ISCMAK_34_TWN_1.0X1	14	17	17	2	OPACO
ISCMAK_42_TWN_1.2X	14	13	14	3	OPACO
ISCMAK_46_TWN_3x4	0	0	0	5	OPACO
ISCMAK_47_TWN_1.2x	15	13	13	2	OPACO
ISCMAK_50_TWN_1.2X	16	16	16	4	OPACO
ISCMAK_52_TWN_1.0X1	18	21	21	1	OPACO
ISCMAK-39_1.2X	19	18	18	3	HIALINO
ISCMAK_03_USA_2.x	0	0	0	4	HIALINO
ISCMAK_04_USA_2.x3	9	9	9	4	OPACO
ISCMAK_05_USA_3x1	1	1	1	5	OPACO
ISCMAK_08_USA_2.x	7	7	7	4	OPACO
ISCMAK_09_USA_2x1	7	8	8	3	OPACO
ISCMAK_12_USA_2.5x	3	3	3	4	OPACO
ISCMAK_14_USA_2.5x1	2	2	2	5	OPACO
ISCMAK_27_USA_1.5x2	9	8	8	3	OPACO
ISCMAK_28_USA_2x1	8	8	8	4	OPACO
ISCMAK_32_USA_2.x	4	4	4	5	HIALINO
ISCMAK_36_USA_2.x	9	7	7	1	OPACO
ISCMAK_38_USA_3x1	0	0	0	5	OPACO
ISCMAK_43_USA_2.5x	0	0	0	5	OPACO
ISCMAK_48_USA_2.x2	9	8	8	3	HIALINO
ISCMAK_51_USA_2.5x1	0	0	0	5	OPACO
ISCMAK_54_USA_2x1	3	3	3	4	OPACO
ISCMAK_57_USA_2.5x1	7	7	7	4	OPACO
ISCMAK_63_USA_3x	1	1	1	5	OPACO
ISCMAK_64_USA_1.5x1	9	9	9	4	OPACO
ISCMAK_66_USA_2.5x	1	1	1	4	OPACO