REPORT OF THE THIRD SHARK AGE AND GROWTH WORKSHOP

SPONSORED BY

THE INTERNATIONAL SCIENTIFIC COMMITTEE FOR TUNA AND TUNA-LIKE SPECIES IN THE NORTH PACIFIC

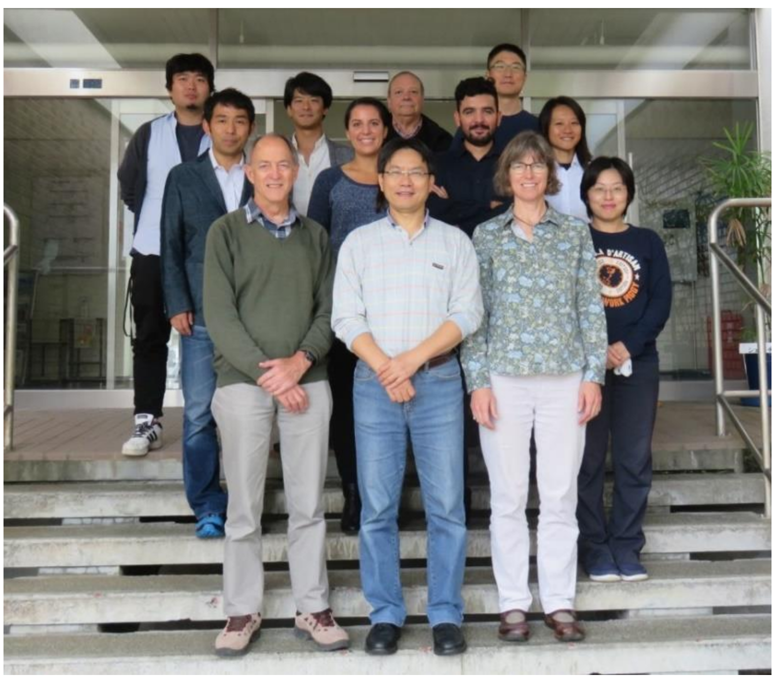
19–24 October 2017
National Research Institute of Far Seas Fisheries
Shizuoka, Japan

1. Opening of the Age and Growth Workshop

Dr. Mikihiko Kai, Acting Chair of the ISC SHARKWG (vice chair of ISC SHARKWG), welcomed the participants to Shimizu and opened the meeting.

Dr. Hideki Nakano, Director of the National Research Institute of Far Seas Fisheries, Shizuoka Laboratory, welcomed workshop participants with greeting remarks.

Thirteen scientists from Chile, Chinese Taipei, Japan, Mexico, New Zealand, and USA participated (Attachment 1).



A draft agenda was reviewed and finalized (Attachment 2). Attachment 3 provides a list of papers and presentations.

Mikihiko Kai, Yausko Semba, and Suzy Kohin facilitated the meeting. The following participants served as rapporteurs for sections of the report.

Section	Rapporteurs
2	Julianne Taylor, Suzy Kohin
3	Enzo Acuña, Suzy Kohin, Yasuko Semba
4	Julianne Taylor, Malcolm Francis, Suzy Kohin
5	Minoru Kanaiwa, Suzy Kohin
6	Malcolm Francis, Yasuko Semba
7	Alberto Rodríguez, Mikihiko Kai
8	Kwang-Ming Liu, Suzy Kohin, Yasuko Semba
9	Shan-Hui Su, Yuki Fujinami

2. Overview of meeting objectives and confirmation of goals

General introduction of growth of North Pacific shortfin mako (*Isurus oxyrinchus*)

Yasuko Semba presented the growth parameters of shortfin mako (*Isurus oxyrinchus)* known in the North Pacific and described detailed known information, including area covered, sample size, material, size range of samples, enhancement method, validation/verification method, and the hypothesis of band pair (bp) deposition. Currently, there are intra- and inter-regional differences in the growth rates of these populations. For an intra-regional example, a study in the Western and Central North Pacific (WCPO) was introduced because the populations were large compared to that within the Eastern North Pacific Ocean (EPO). Although the total area covered and the overall size range of samples were similar, sample sizes for each size class were much lower in the EPO (especially for samples between 70 and 100 cm precaudal length; Semba et al. 2009). Furthermore, the uncertainty of the periodicity of growth bp formation for small juveniles (≤100 cm PCL) was noted. With respect to inter-regional differences, it was noted that validation (Wells et al. 2013; Kinney et al. 2016) and verification (Ribot-Carballal et al. 2005) are both available with varying results, depending on the study, but that no sex-specific growth curve has been estimated based on either bp counts or other sources (e.g., size data analysis and tag-recapture data). For the latter, the necessary information for obtaining length-at-age relationships was briefly introduced, which are the periodicity of bp deposition, the month in which growth bands are formed, and the birth month.

Prior ISC SHARKWG efforts on shark age and growth

Suzy Kohin provided a summary of the past efforts of the ISC SHARKWG regarding shark age and growth. The focus of prior work has been on shortfin mako and blue sharks, which are the two species assessed by the SHARKWG. Since 2011, the SHARKWG has sponsored two workshops and several webinars. The overarching goal of the work of the shark aging specialists has been to understand differences in the results of previous ongoing aging studies to compare and combine age readings to derive the most plausible growth information for blue and shortfin mako sharks. A work plan was developed and a reference collection of vertebrae was collated to corroborate age readings among readers and address age reading variability between studies. To date, aging specialists from Japan, Mexico, Taiwan, and the USA have provided results from cross-readings of x-rays, processed in the USA, of Oxytetracycline (OTC) age validation of vertebrae (referred to as Dataset A) and an ISC reference collection of vertebrae images processed by x-ray (Dataset B). Results from the processing of reference vertebrae from individual labs using various methods (Dataset C) will be discussed during this meeting. A meta-analysis that integrates the results of several aging studies in a Bayesian framework will be used to provide growth curves for the upcoming North Pacific shortfin mako shark assessment. Issues regarding vertebral age readings that are important to resolve during this workshop include 1) the interpretation of translucent/opaque bands and edges (in comparison to thick/thin, concave/convex, hypo-mineralized/hyper-mineralized, etc.) and how to use edge readings in age estimates; 2) timing of the birth month and how it is used to adjust age estimates; and 3) incorporation of data and uncertainty in the meta-analysis.

A potential method for a meta-analysis of the growth curve of the shortfin mako shark in the North Pacific

Norio Takahashi provided a summary of the proposed Bayesian meta-analysis that effectively utilizes all age and growth data provided by the ISC Shark Working Group members to estimate a growth curve for shortfin mako sharks in the North Pacific Ocean. The methods are described in Takahashi et al. (2016). The method of meta-analysis to estimate a growth curve was reviewed and the usefulness of this method was briefly examined using simulated age and growth data for hypothetical fish species. Points/issues to be considered or resolved before applying the method to these data were raised. Participants were asked to provide the data needed for the meta-analysis during this workshop so that candidate growth curves could be estimated.

Discussion

Some of the issues raised regarding age reading were discussed, including the importance of knowing the birth month and when band formation occurs (necessary for estimating length-at-age). Based on Japanese vertebral aging studies (Semba et al. 2009), formation of the birth band occurs shortly after birth, and the first band is formed in the winter (December) with the deposition of a single bp per year. Parturition was estimated to occur in the spring. These results were discussed further after reviewing information from other studies.

Another issue raised was the interpretation of bands. Building a translation table for each study/method to denote differences in reading band pairs (bps) was discussed. For each data set submitted for the meta-analysis, notes should be made for how vertebrae are read and processed to ensure accuracy of the data and analysis.

Regarding the regional differences in growth, stock structure was briefly discussed. The ISC SHARKWG considers there to be a single stock of shortfin mako in the North Pacific, distinct from that of the South Pacific, based on genetics and tagging data (Sippel et al. 2011; Urbisci et al. 2013; Michaud et al. 2011; Taguchi et al. 2015). It was acknowledged that shortfin makos are found near the equator, and there is a single report of a tagged shortfin mako moving from off the coast of Australia into the North Pacific equatorial waters (Bruce 2013). Furthermore, while shortfin makos tagged in the Northeast Pacific have been recaptured in the Northwest Pacific, satellite tagging data suggests that movements of shortfin makos may be predominately confined to smaller regions, with few individuals making broad-scale migrations. Because of limitations in the existing tagging and genetics studies, continued review of emerging information is recommended to refine stock structure assumptions.

Regarding the meta-analysis, how to incorporate uncertainty among studies (differences in estimated growth among studies) and uncertainty within studies (based on confidence in reading individual vertebra samples or in growth curve estimates) was discussed. Although ideally such detailed uncertainty information would be incorporated, it is not yet clear how those data can be used. If there is information regarding age reading confidence for individual vertebrae, participants were asked to provide those. While the meta-analysis uses the von Bertalanffy growth function (VBGF), the modellers believe that growth curves estimated using other equations could also be used. However, more complicated models may not converge. It is preferable to try the model first with growth curves estimated using the VBGF. The potential for using growth previously estimated from length frequency (LF) data, e.g., data from Kai et al. (2015), in addition to using growth curves based on vertebrae was discussed. Length frequency (LF) studies can likely be used, with the consideration of how to weight those studies relative to the vertebral studies, particularly, given the typically larger sample sizes associated with LF studies.

3. Review of ongoing work (cross-reading among national scientists)

<u>Cross-readings for shortfin mako shark reference vertebrae among USA, Japan, and Mexico</u>

Julianne Taylor (JT) provided a summary of the results from the independent processing of reference collection vertebrae and cross-readings (Dataset C). Reference collection vertebrae were originally processed by Michael Kinney (MK) using the hard x-ray methodology. A second reader from the USA (JT), as well as readers from Japan and Mexico, independently read and aged 61, 48, and 63 vertebrae, respectively, from the ISC reference collection. JT followed the same criteria with MK and their results were evaluated independently. Results were compared using the original USA readings (MK) as a standard. Each country used its own method: those from

Japan used the surface shadow method, and those from Mexico used thin sections with transmitted light. The results from the USA (JT) counts had high exact agreement at 62.3% and within one bp agreement at 91%. Agreement with results from Mexico was not as high with 23.8% exact agreement, and within one bp agreement at 40%. Although these percentages seem low, Mexico's counts were not significantly different from the counts by MK. Japan's counts had low exact agreement at 14.6% and within one bp agreement at 66%. The shadowing method used in Japan showed a significant negative bias (lower counts) with a *p*-value of 0.007. Edge readings for each country varied slightly. Results from the USA (JT) and the USA standard (MK) had a high agreement on edges at 91.7%. Notably, researchers from Mexico and Japan read more translucent edges than MK, with 78.7% and 72.6% agreement, respectively.

Measurements analyzed included the distance from the focus to the distal edge of the birth band and the total length from the focus to the centrum edge. Among all countries, size measurements were more variable for older sharks. Measurements by researchers in Japan were consistently smaller than MK's measurements. In general, the USA (JT) and Mexico measurements were consistent with those by MK. Measurements to the distal edge of the birth band showed more variability with younger sharks across all countries. Overall, there was no significant difference or high variability with this measurement.

<u>Discussion</u>

These results indicate that fewer bps are identified using the surface shadow method than using methods applied by scientists from the USA and Mexico. Band pair counts between the scientists from the USA and Mexico were almost similar. Inter-reader differences based on the same enhancement method (x-ray, Dataset B) were much smaller than differences derived from different enhancement methods (Dataset C). That is, different enhancement methods lead to different bp counts. It was pointed out that despite reading fewer bps, after converting Japan's bp counts to ages, there will be smaller differences in the estimated growth curves. This is because Japan verified bp deposition periodicity at one bp per year by centrum edge analysis. Japan indicated that they were unable to process most of the vertebrae provided by Taiwan because the surfaces appeared damaged, perhaps due to a cleaning process.

It was also noted that Taiwan uses a soft x-ray method for aging shortfin mako shark vertebrae. To better understand how their bp counts may differ from the other methods used, Taiwan also planned to process the reference vertebrae, but they have not yet done so. Nevertheless, Taiwan's shortfin mako age and growth study could be useful in the meta-analysis if the individual age and shark size data are available.

One growth curve, based on the reference collection of vertebrae, should be useful in the meta-analysis. Members agreed to use a growth curve based on MK's readings in the meta-analysis. Ages will be derived by applying a bp periodicity of two bp/y for the first ten bps and one bp/y thereafter.

4. New information on the aging of shortfin mako, with regional updates

<u>Standardization of mako shark aging through different vertebrae enhancement methods and comparison of growth estimations from Eastern and Western North Pacific Ocean</u>

Alberto Rodríguez presented the preliminary results of a collaborative work on age and growth of shortfin mako shark in the North Pacific Ocean (NPO) between Mexico and Japan. The aim of this collaboration was to standardize the aging criteria through cross-reading comparisons of growth band counts using different methods of enhancement readings on vertebrae. The standardization was used to develop consensus age and growth curves of shortfin mako shark in the NPO. This study compared bp counts derived using two methods applied to a collection of vertebrae obtained in Mexican fisheries: 1) surface shadow (as in Semba et al. 2009) and 2) hard x-ray (as in Wells et al. 2013). The results indicated that that fewer bps are read with the surface shadow method. For the hard x-ray method, ages were estimated by assuming a bp deposition rate of two per year for the first five years and one per year afterwards. For the vertebrae examined, a similar growth curve was produced using counts derived from the surface shadow method, assuming one bp deposition per year.

Discussion

Observed lower counts using the surface shadow method is consistent with the analysis presented above for the ISC reference collection (Dataset C). Notably, the samples are from a region within the California Current, known to be frequented by shortfin makos that range continuously along the western coasts of the USA and Mexico in the eastern Pacific. Thus, based on these results, members agreed that applying a hypothesis of two bp/y for five years and one bp/y thereafter is appropriate to estimate ages for EPO mako sharks using the three latter enhancement methods.

However, it is important to note that in any study, knowing the true ages and whether bp counts can be directly translated into ages is not possible (i.e., it is not clear which, if either, set of results is indeed correct). Other data sources need to be considered when determining the best hypotheses, such as LF data (Kai et al. 2015), bomb radiocarbon validation, tagging data, and the maximum longevity of greater than 30 y (which has been observed in some studies using various methods).

For the meta-analysis, the first step is to obtain bp counts and plot these against length. Otherwise, results may be confounded with hypotheses about band deposition. Subsequently, the most plausible hypothesis (es) regarding bp deposition can be applied to the data to generate ages and, potentially, new VBGFs.

Members discussed the potential for differences among and within studies depending upon the location of vertebral collection. It is believed that location on the vertebral column does not likely affect bp counts, but it could affect back-calculation of length-at-age if a mixture of the cervical and trunk vertebrae are used.

Update of growth of juvenile shortfin mako (*Isurus oxyrinchus*) in the Western and Central North Pacific Ocean.
Yasuko Semba presented an update on recent research regarding the growth of juvenile shortfin mako. The growth patterns of shortfin mako (*Isurus oxyrinchus*) vary depending upon the location of the study, both within the North Pacific and among oceans. In the Western and Central North Pacific Ocean (WCPO), the growth rate based on total length data of juveniles (smaller than approximately 150 cm precaudal length [PCL]) was faster than that based on vertebrae. Between WCPO and the Eastern North Pacific Ocean (EPO), a different hypothesis of periodicity of bp deposition has been proposed. The disagreement of growth within WCPO was further investigated by analyzing vertebral bps for a greater number of small juveniles (≤100 cm PCL), which were scarce in the former study. The results support annual bp periodicity for small juveniles. They also suggest that differences in growth rates between studies could be explained partly by the aging of additional samples within the size range between 70 and 100 cm PCL. Filling the gap for this size range, as well as applying several approaches other than bp counts (e.g., size frequency data analysis, tag-recapture data, and chemical analysis of centrum), will improve the accuracy of estimated growth parameters for this population.

Discussion

The additional ages for sharks <100 cm PCL provides a growth curve with faster early juvenile growth, but still not as fast as for the LF data reported by Kai et al. (2015). It was agreed that this revised growth curve should be used in the meta-analysis.

It is possible that birthdate assumptions vary across studies. Thus, for each study, it is necessary to correct calculated ages based on the most appropriate birthdate estimates, which can be determined from the best available information (such as embryonic development or LF data). How bps and edges are read when compared to x-rays and light microscopy images was also discussed. Each member was queried regarding vertebral bp imaging for their studies. A translation table for bps and edges is provided in **Table 1**. If the edge is wide, then the partial year is added from the last growth band. Specific details regarding how to account for partial bands on the edge are treated differently among studies.

Table 1. Summary of the translation for band pairs and edges for each national delegation

	Method	Wide	Narrow	Band Pair	Treatment of birth band
US	Hard x-ray	Translucent	Opaque	Wide/Narrow	Not counted
Japan	Shadowing	Concave	Convex	Concave/convex	Counted
Mexico	Transmitted light	Opaque	Translucent	Wide/Narrow	Not counted
Taiwan	Soft x-ray	Translucent	Opaque	Wide/Narrow	Not counted
New Zealand	Reflected light	Opaque	Translucent	Wide/Narrow	Not counted

Estimation of growth curve from length composition of juvenile shortfin mako, *Isurus oxyrinchus*, in the Western and Central North Pacific Ocean

Mikihiko Kai presented an estimation of growth from the length composition of juvenile shortfin mako in the Western and Central North Pacific Ocean using port sampling data collected from 2005 and 2013 (Kai et al. 2015). The monthly length compositions show a clear transition of three modes in the size range smaller than 150 cm precaudal length (PCL); this is believed to represent the growth of age-0 to age-2 classes. These classes were then decomposed into age groups by fitting a Gaussian mixture distribution. Simulation data of lengths at monthly ages were generated from the mean and standard deviation of each distribution, and fit with a von Bertalanffy growth function. Parameters of the estimated growth curves for male shortfin mako were 274.4 cm PCL and 0.19 y^{-1} for L_∞ and k, respectively, while parameters for female shortfin mako were 239.4 cm PCL and 0.25 y^{-1} for L_∞ and k, respectively. This indicates apparently faster growth than previously reported. We also observed that juvenile shortfin mako less than 66 cm PCL were mostly caught in the waters near Japan in late winter to early spring. Thus, we further updated the growth curve using the length frequency data from 2005 to 2016. The estimated growth curve for the combined data was similar to that of the previous study (Kai et al. 2015). The new analyses indicated that the shortfin mako of small sizes, i.e., less than 70 cm, were mainly collected in Feb, Mar, and Jun. These results suggest that the primary birthing seasons are in winter and spring and may occur a few months earlier than reported in the Western and Central North Pacific Ocean. Overall, the analyses support the previously reported birth size of 59–60 cm in PCL, as proposed by Semba et al. (2011).

Discussion

Fish below 70 cm PCL are close to newborn size, suggesting that birth may occur over an extended period (ca. 6 months). Results are possibly confounded by a spatial shift in the fishing fleet as the targeted fishing region changed through time. Thus, caution must be taken with the interpretation of LF proportions.

Fitting mixtures to data (e.g., MULTIFAN) usually underestimates the number of cohorts and overestimates growth, especially when modes become indistinct. Thus, it is best to confine use of LF data to 0+ and 1+ modes (i.e., cut-off at 24 months). It was agreed that a subset of the simulated LF frequency data that was classified by month, up through month 24, would be provided to weight data appropriately in the meta-analysis.

Application of EPMA to juvenile shortfin mako collected in the Western and Central North Pacific Ocean

Yasuko Semba presented an application of EPMA (Electron Probe Micro Analyzer) to juvenile shortfin mako. Generally, growth bands have been assumed to reflect variation in the environment throughout a year, as the growth increment is large in summer and small in winter with compositional changes in each increment (hereafter, indicated as "band"). However, there is no known study that addresses the chemical composition in each band for shortfin mako. In this document, the preliminary results of EPMA (Electron Probe Micro Analyzer) analysis were introduced. EPMA was conducted to check if the band assigned in traditional aging could be distinguished as an area with a different chemical composition and if each structure corresponds with each other. In the current analysis, (1) the method described in Cailliet and Radtke (1987), and (2) a simplified method (linear analysis) were both applied to the corpus calcareum in the cutting plane, while (3) linear analysis was applied to the centrum surface excised from same specimen; in this way, the occurrence pattern of several elements (mainly Ca and P) were estimated. For the analysis of (1) and (2), no clear patterns of occurrence were discerned from the outer edge to the core, but a clear periodic pattern of occurrence was observed in the analysis of (3). For the analysis of (3), the overlap between the compositional image in the backscattered electron mode and the occurrence pattern of the main elements were estimated using EDS (energy dispersive spectroscopy) analysis. The reason why a different pattern was observed depending on the method is unclear, but the electronic and compositional images suggest many void structures in the section along the corpus calcareum. Although further research is required to determine the potential effectiveness of this method as a tool for objectively estimating the number of bps, as well as considering the periodicity of bp deposition, the current analysis suggests that linear analysis has greater potential to detect the occurrence patterns of main elements across the time series.

Discussion

Differences between surface scan results and corpus calcareum scans on the cross section were discussed. The former shows in-phase cycles in Ca and P, with an inverse cycle in C, whereas the latter shows no consistent pattern. It is not clear why different parts of the centrum produce different results. Because magnification reveals voids in the vertebrae sections, it may be that the analysis is less accurate because it identifies areas which lack mineralization. The cycles in chemical composition may correspond to banding, which are seen as hypo- and hyper-mineralization in vertebrae; however, such banding patterns have not yet been examined in this species. Thus, this approach appears to be the next step and, if successful, may prove to be a useful, objective way to identify bands. This was a pilot study with a single sample, but the surface scan analyses look promising. The time and cost were not considered prohibitive.

Another method (scanning x-ray fluorescence microscope [SXFM]; Raoult et al. 2016) was mentioned, but further thought will need to go into the biological interpretation of potential causes of any cycles (e.g., age, migration, or freshwater input). The time frame required for development of either using EPMA or SXFM is likely too long for these methods to be useful for the present exercise; however, members were encouraged to pursue these types of studies if possible.

Size, maturity, and age composition of mako sharks observed in New Zealand tuna longline fisheries

Malcolm Francis presented size, maturity, and age composition data for mako sharks in New Zealand. This study assesses the catch composition of mako sharks taken by surface longline (SLL) in New Zealand waters using data and samples collected by observers. Data were stratified by fleet (chartered Japanese or New Zealand domestic vessels) and region (North and Southwest). Length-frequency distributions were scaled up to estimate the size composition of the commercial catch for the fishing years 2007 to 2015. Vertebrae were sectioned, and growth bands were counted to estimate the age of a subsample of sharks. An aging protocol was developed, and growth curves were fitted to the length-at-age data. The SLL mako catch was dominated by juveniles, with most sharks shorter than the 200 cm fork length; overall, 89% of males and 99.5% of females were estimated to be immature. However, these proportions may be over-estimates if significant numbers of large mature adults were discarded unmeasured. The 0+ age class constituted about one-quarter of the catch, and most of the catch was less than 6 y old. There is an urgent need to validate mako aging to determine whether one or two bps per year are deposited on their vertebrae. Such validation could be achieved by the injection of oxytetracycline into tagged and released sharks to mark their vertebral centra with a time stamp. Assuming that one bp per year is deposited, males and females grow at similar rates up to about 16 y, beyond which there were few aged sharks.

Discussion

The very high growth rate inferred from the LF distributions, which more closely corresponds to the growth estimate that assumes a deposition rate of two bp/y, was unexpected. This is similar to the rapid growth of juveniles observed in the LF data from the NWPO (Kai et al. 2015), as well as in other studies. Across studies, growth rate estimates from LF appear to be higher than growth rate estimates from vertebral band studies. It was noted that different length metrics are used among studies; for comparative purposes and for the ISC assessment, there is a need to finalize and agree on PCL conversions. Growth information, based on LF data from several studies, was combined and examined graphically to determine whether there were consistencies among studies. LF growth was quite consistent across several regions; members considered the information on growth from LF modes to be more representative than growth based on vertebral bp counts for younger sharks (**Figure 1**). A difference in length at birth was noted for mako sharks from New Zealand (approximately 61–62 cm FL) and the North Pacific (the former being lower [after conversion to PCL], but this difference was based on backward extrapolation of free-living individuals to a theoretical birth date [October 1] and only one litter of embryos from a pregnant female).

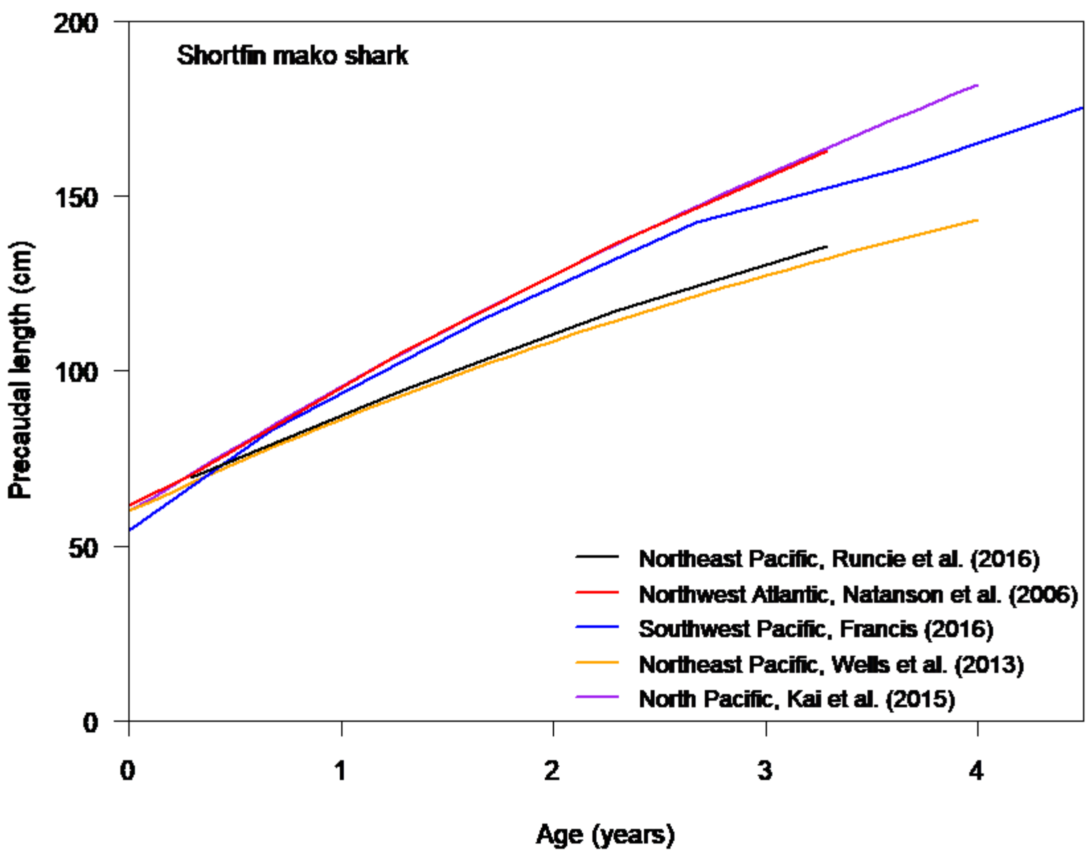


Figure 1. The growth curves based on the analysis of size-frequency data for mako sharks in the Pacific. Wells curves come from the parameters provided by Kai et al. (2015).

Age and growth estimates of the shortfin mako shark, *Isurus oxyrinchus*, in the Northwest Pacific

Kwang-Ming Liu and Shan-Hui Su presented previous work on the age and growth estimates of the shortfin mako shark. Age and growth curves of the shortfin mako shark, *Isurus oxyrinchus*, were described from 348 specimens

(215 females and 133 males, 85–375 cm in total length). The specimens were collected from October 2001 to March 2003 and caught by a Taiwanese small scale longline fishery in the Northwest Pacific. A single growth bp (comprising translucent and opaque bands) formed each year, with up to 31 and 24 bps observed for females and males, respectively. Marginal increment ratio analysis indicated that the opaque band formed from July to August. As the pupping season was assumed to occur in March, the first opaque band deposition was estimated to begin at 0.42 y. Four growth functions were used to fit the observed length-at-age data: the von Bertalanffy (VBGF), the two-parameter VBGF, the Robertson, and the Gompertz functions. The sex-specific growth equations significantly differed. Based on Akaike's information criterion, the Gompertz function and VBGF provide the best growth functions for females and males, respectively. The parameters of the Gompertz function were estimated as follows: L_∞ = 485.7 cm TL, growth coefficient (k) = 0.052 y^{-1} for females. The parameters of VBGF were estimated as follows: L_∞ = 332.1 cm TL, k = 0.056 y^{-1}, t_0 = -6.08 y for males.

Discussion

The revised VBGF from the present study should be used in the meta-analysis rather than that from Chang and Liu (2009).

Growth estimation update of the shortfin mako shark in the Mexican Pacific Ocean, through multi-model approach and different methods for age determination

Alberto Rodríguez presented updated age and growth estimates of the shortfin mako shark caught in the Mexican Pacific Ocean, for the periods 2001–2003 and 2008–2016. Sample size was higher than in a previous study in the region (Ribot-Carballal et al. 2005), plus add new growth band periodicity information on vertebrae (biannual deposition). Centrum edge analysis (CEA, Okamura & Semba 2009), for whole vertebra stained with silver nitrate, and marginal increment analysis (MIA, Okamura et al. 2013), for sectioned vertebrae, were used to determine the periodicity of growth band formation. These results were compared with information from direct validation studies. The traditional von Bertalanffy growth equation (vB), Gompertz, and logistic models were fitted to the data set, and model performance was assessed with small sample bias-corrected Akaike information criterion (AIC_c), Akaike differences (Δ_i) and Akaike weights (w_i).

A total of 256 individuals (centra and sectioned combined), corresponding to 130 females (total length, LT: 65–302 cm) and 126 males (TL: 64–267 cm), were examined. The precision of growth band counts was acceptable, and whole vertebrae produced slightly more precise counts (PA= 70/62, APE= 4.3/5.6, CV= 6.2/7.9) than sectioned vertebrae (PA= 76/37, APE= 4.5/14.5, CV= 6.4/20.5) for intra and inter-reader comparisons, respectively. The CEA and MIA suggested biannual bp formation for juveniles; thus, age was estimated by assuming growth bp deposition rate of two per year until first five years and one per year afterward. The estimated ages ranged from 0 to 15 for females and 0 to 12 for males (centra and sectioned pooled). AIC values estimated with the Gompertz function were slightly better than those estimated with either the vB or logistic functions for both females and males. The vB was chosen, however, as the best model due to the more accurate prediction of life history values, compared to those reported for shortfin mako in recent studies. Thus, the von Bertalanffy function was used to estimate the following growth parameters: L_∞= 365, k= 0.11 and t_0= -2.03 for females and L_∞= 298.7, k= 0.15 and t_0= -1.76 for males. This study contributes to knowledge of the vital life-history parameters of shortfin makos. The growth parameters can now be incorporated into the future ISC stock assessment models to assist science-based fishery management and conservation initiatives.

Discussion

The data were not corrected for birth month but will be before the meta-analysis. There was some overlap in samples with Ribot-Carballal et al. (2005), but the preferred method of Mexico now is different. It was recommended that a single set of results for all the samples from Mexico (with only the new readings for Ribot-Carballal et al. (2005)'s specimens) be provided for the meta-analysis.

Tentative birthdate of shortfin mako suggested by length compositions collected from a Mexican fishery

Alberto Rodríguez presented a number of size data by year and month that were analyzed from the length composition of shortfin makos caught in waters near Mexico from 2006 to 2016. The main purpose of this analysis was to examine the birth month (season) and birth size of shortfin mako caught in the Eastern Pacific Ocean (EPO). The differences between precaudal lengths (PCL) that were calculated by ISC delegations were compared. The results indicate that the Mexican fishery catches the smaller sizes of shortfin mako, including those smaller than 60 cm PCL. The presence of pups less than 70 cm PCL occurs from March to September (with the highest frequency caught in July and August). The smallest sizes caught in this study range from 55 to 60 cm PCL, which closely resembles the average birth size (59–60 cm PCL) calculated in the Western North Pacific. Moreover, the graphical comparisons between PCL estimates, generated through relationship equations by each ISC delegation, indicate that there were no differences between regions when the length for smallest sharks is calculated. From these data, based on the reproductive and growth studies in Mexico, a tentative birthdate of June 1st could be assumed.

Other recent relevant studies

An ICCAT working paper (Rosa et al. 2017) provides updated growth curves for male and female shortfin makos in the North Atlantic based on vertebral band counts. Prior studies had lower sample sizes and were focused on smaller geographic regions. The estimated growth curves were considered the best available for the North Atlantic and were used in the recent ICCAT mako assessment. The authors observed "shadow" bands between "true" bands for the first five years; thus, they suggest that other studies that have concluded a vertebral bp deposition rate of two bp/y may be reading "shadow" bands as "true" bands, at least for juvenile sharks, thus, providing higher counts. If so, resultant growth curves would be similar when assuming one bp/y when processing and counting, as in the North Atlantic, versus assuming two bp/y for five years when processing and counting, as in the EPO.

A recent review paper (Harry 2017) looked at a large number of elasmobranch age validation studies (bomb carbon and OTC) to try to determine the reliability of using vertebral bp counts to estimate age. Age is likely to have been underestimated in a large number of studies across species, including 50% of those validated using bomb carbon dating. Ages were typically underestimated in larger and older individuals. The author suggests that age underestimation is likely a systemic issue associated with the current methods and structures used for aging. A potential underestimation of longevity, may unexpectedly bias growth and mortality parameters, due to the apparent loss of population age-structure. Comments regarding this paper have pointed toward a need to find alternative methods for aging sharks and moving away from using vertebral bps.

Discussion

Members discussed the latter study by Harry (2017); although there is a widening belief that vertebral bands may not reliably provide robust age estimates for all shark species, the study highlights that the problem of age underestimation is greater for larger and older sharks. This working group has completed a number of comparative studies to at least corroborate age readings across studies and examine alternative data, such as length frequency and tagging data. The group believes that the meta-analytical approach, which incorporates many prior studies including LF data that appear to show relatively rapid growth, will provide the best available information for the upcoming assessment; however, continued research is recommended to improve current estimates and/or find alternative approaches to aging shortfin mako and blue sharks. It was also recommended that such a study might take advantage of the Fukushima radiation signal. Fukushima radiation signals are already being used to track migration in bluefin tuna, but members are not aware of any current shark studies using this signal.

Following all presentations about the updated and recent results, data from a number of studies were identified for potential use in the meta-analysis (**Table 2**).

Table 2. Summary of the studies identified for potential use in the meta-analysis

Mexico	A study introduced in this workshop (Rodriguez-Madrigal et al.) after data are corrected for birth date and capture date
US	LF data from shark survey in Runcie et al. (2016); Vertebrae data from Wells et al. (2013), Kinney et al. (2016) and Lyons et al. (2015)
Taiwan	An updated study included in this workshop (Liu and Su)
Japan	Simulated LF data for ages 0–24 mo (Kai et al. 2015); An updated study included in this workshop (Semba et al.)

5. Estimation of age from the number of growth band pairs (difference of band pair periodicity)

The assumptions that are necessary to obtain length-at-age (i.e., converting bp readings to ages) were discussed. Regarding the periodicity of growth bp deposition, members agreed that each study adopts the periodicity hypothesis which fits best in each dataset considering that cross-reading experiments demonstrated differences in visualization of bps across studies. Thus, Japan and Taiwan should adopt the annual hypothesis; in contrast, the US and Mexico should adopt the hypothesis of two bps per year until 10 bps (i.e., for the first five years) with a transition to a single annual bp thereafter (hereafter referred to as the "two to one bp transition hypothesis"). It is still important to verify whether annual bps are seen for younger age classes based on the soft x-ray method of Taiwan, but their method has not yet been compared in cross-reading experiments. Regarding the birth month, Japan, Taiwan, US, and Mexico will adopt May, March, May, and July, respectively, to take into account the possible spatiotemporal variation of pupping. Regarding the month in which growth bands are formed, Japan and Taiwan will adopt December and August, respectively. However, the assignment of month of bp deposition is more difficult for those adopting the two to one bp transition hypothesis.

For data from Japan, the estimation of length-at-age is derived from summation of 1) the difference in the period between the 1st month (i.e., birth month) and the month in which the growth band deposits; 2) the year (number of bands minus 2); and 3) the difference between the capture month and the month of growth band deposition from the annual hypothesis. In this case, the first band (birth band) is counted so the "bp count minus two" is the number of years minus the first two bands that are formed in the first year (i.e., the birth band and the 1st full growth band). Taiwan has a different assumption regarding the first growth bp and assumes that the age of sharks with a complete growth bp is age class one.

For the EPO where a transition from two to one bps is assumed after five years (10 bps), estimation of ages is derived from the sum of 1) the number of bps (counted distal to, i.e., not including, the birth band) divided by two for the first 10 bands plus the sum of the number of bps distal to the 10th bp; and 2) the difference between the capture date and either the birthdate (if number of bps is even or greater than 10) or the birthdate plus six months (if the number of bps is odd and/or less than 10). As noted during the 2nd ISC Age and Growth Workshop, the method of bp reading in Wells et al. (2013) differs from that adopted by the ISC and subsequently by the US scientists. The current age reading manual describes the current method in use for the ISC reference collections and by the US. When comparing readings from Wells et al. (2013) with those in other studies, the different method for reading bps should be taken into account.

6. Other life history parameters for stock assessment

<u>Biological parameters necessary for the stock assessment of shortfin mako in the North Pacific</u>

Mikihiko Kai presented the biological parameters necessary for the stock assessment of shortfin mako in 2018 and listed them, assuming that both a production model (BSPM) and age-structured model (SS) are used. The list includes parameters on growth, reproductive biology, length-weight (L-Wt) relationships, natural mortality, productivity, shape parameters, and a stock-recruitment relationship. Some candidates for the estimation method and plausible values for the first four terms (growth, reproductive biology, L-Wt relationships, and natural mortality) were discussed; the latter three parameters are strongly related to and dependent upon the first four.

Discussion

Birth length (±SE), estimated by fitting growth curves for Taiwanese and Japanese embryo data, indicated a poor fit for some litters; thus, the effect of arbitrary assumptions of mating period was discussed. The values of 60 cm and 6 cm PCL can be used as mean and SD, respectively, for the prior distribution based on the analysis by Kai et al. (2015).

Regarding longevity, Taiwan observed maximum ages of 24 y for males and 31 y for females. Mexico observed maximum ages of 12 y for males and 15 y for females. Observed maximum age values may underestimate longevity and theoretical estimates may overestimate longevity (Cortés 2000; also refer to the longevity column comparison of empirical and theoretical values in the Appendix). Ardizzone et al. (2006) applied the bomb radiocarbon chronologies to shortfin mako and validated the maximum age as 31 y. It was agreed that this value is a reasonable estimate of longevity for the shortfin mako. It is necessary to derive the growth parameters in the meta-analysis before longevity can be estimated using theoretical calculations.

For breeding periodicity, the variability of gestation period and uncertainty regarding the resting period were discussed. As we cannot currently obtain reliable estimates, especially for the resting period, using alternate scenarios or a relative weighting of the hypothesized 2-y and 3-y reproductive cycles was proposed.

For sex ratio at birth, 1:1 was approved, and the relationship between maternal size and litter size was discussed. For the latter, the Japanese study supports a linear relationship while the Taiwanese study does not show a clear trend; thus, it was requested that the relationship be re-examined after combining the data. Observed litter sizes may underestimate actual values because of premature abortion.

For length at maturity, there are large differences in L50 between both sexes from the Japanese and Taiwanese studies. It was suggested that the data should be combined, along with the data from Mexico and that the maturity values be recalculated.

There is a need to have sex specific L-Wt relationships for the assessment. Each national delegation that has L-Wt data will provide those (in PCL and kg) for comparison and potential use.

Regarding natural mortality, there are insufficient tagging data to directly estimate M (i.e., direct method). Therefore, an indirect method (for example, using an empirical equation based on the estimated life history parameters) must be used. It is likely that age-specific and sex-specific M's will be needed, but age-related changes are not expected to be large because makos are large at birth. Options for estimating M will be discussed during the next webinar.

7. Discussion about the meta-analysis (and candidate growth curves to be used)

Characteristics of the raw age and growth data compiled and shared by each delegation were reviewed, and points/issues to be considered or resolved before applying the data were raised.

Discussion

The Japanese delegation gave a brief explanation about their age data, assuming May as the birth month and December as the month of growth band formation. Considering an annual bp deposition, ages were estimated as described in Section 5 above. It was recommended that Japan add 0.5 mo to each age so that sharks born in May would be considered 0.5 mo old.

Other delegations also explained their calculation methods for length-at-age for each sex for the meta-analysis.

The discussion also included whether a single birth month should be applied by all delegations and whether the model could accommodate either negative ages or many "age 0" (i.e., age-0) sharks if the capture date either preceded or was in the assumed birth month. It was concluded that each delegation should estimate ages based on the assumed birth date for each separate area, and negative ages (rather than truncating all derived negative ages to 0) would be preferred when providing data for the meta-analysis. The analysts will assume a prior normal distribution on birth month that will account for birth prior to the birth month. The values of 60 cm and 6 cm PCL will be used as the mean and SD values for the prior distribution, respectively. This is based on the LF data of Kai et al. (2015) and is consistent with the size of near-term embryos and small free-swimming pups in Semba et al. (2011). Most delegations have few age-0 sharks and the issue of negative ages may be minor.

Each delegation was asked to upload their set of size-at-age data (whether preliminary or final) into the meeting dropbox for the meta-analysis. The data requested are study, length (PCL in cm), sex, age, capture month, and confidence score. In addition, each member was requested to provide a description to Semba of how the bands were counted and converted to ages for each study for inclusion in the meta-analysis report for the November data preparation meeting.

The US will upload preliminary data for the ISC reference collection readings of Michael Kinney as one study, and preliminary ages for the other US aging studies (Wells et al. 2013; Kinney et al. 2016; Lyons et al. 2015) as a second study. However, they will need more time to finalize the ages in advance of the webinar.

A simulation study was conducted, which assumed that two sets of data among the five datasets would have different estimations (because ages of one dataset were underestimation compared to the ages of the other datasets). These simulated data showed a larger uncertainty surrounding the estimated ages and a slight bias in the median estimation. Another simulation study was conducted as well, which assumed that sharks older than 10 y were removed from the simulation data. The results also showed a slight bias in the median estimation. It was discussed that the measurement error of body length is probably more accurate than the error associated with the estimated age. Thus, it was concluded that it would be better to include the error term associated with the estimated age in the meta-analysis.

Whether any weighting can be applied if some studies are considered more plausible than others was also discussed. The modellers are not yet sure how to do that, but if some data are considered less plausible, the model can be run with and without the data to see their effect. Growth based on LF data from Kai et al. (2015) and from Runcie et al. (2016) were considered more valuable for ages 0–2 y than the vertebral band growth estimates; perhaps there is a way to preferentially use these data in the meta-analysis.

It was agreed that the two-parameter VBGF function be used for the meta-analysis. Seven data sets will be used in the analysis (as shown in Table 2 in Section 4): four delegation growth datasets (JP, US, MX, and TW), one reference collection dataset, and two length frequency datasets (Kai et al. 2015; Runcie et al. 2016).

There was concern about the potential for double counts in the data. Therefore, each delegation needs to carefully check their data and if vertebrae are included in both their reference collection and in the national growth studies; if so, they should perhaps be excluded.

8. Work plan

Members discussed the work needed to provide growth and life history information for the shortfin mako stock assessment and to meet the longer term goals of providing the best growth information on the pelagic sharks of interest to the ISC.

<u>Short term work plan (in advance of the November data preparation meeting)</u>

1. Provide sex-specific L-Wt data. **Within one week**, each national scientist will send length (PCL in cm) and whole weight (in kg) data by sex to Suzan (Taiwan) who will compare the relationships and provide a summary during the November webinar.
2. Provide size at maturity estimates. **Within one week**, each national scientist will send the number of mature/immature sharks in each size class (in 5 cm intervals from 55 cm PCL) or individual size and maturity data, if available for each sex, to Semba who will estimate maturity based on the combined data.
3. Provide information regarding the relationship between maternal size and litter size. **Within one week**, each national scientist will send maternal size and litter size data to Semba who will estimate a relationship based on the combined data.
4. **By the end of the meeting**, for the ISC reference collection, US, Taiwan, and Japan will add the date of collection for each sample provided.
5. Conduct meta-analysis. **By the end of the meeting**, each delegation will upload preliminary age at length data to the meeting dropbox. **Within one week**, each delegation will provide finalized age at length data to Takahashi, as well as a description of the methods used to count bands and estimate ages to Semba. Takahashi will conduct the meta-analysis **in advance of the November data prep workshop**.

<u>Long term work plan</u>

1. Based on the ISC SHARKWG's plans for upcoming shark stock assessments, discuss the needs for each species and priorities for providing age and growth information. Members have archived vertebrae for cross-reading analyses for the blue shark if needed.
2. Research alternative methods for providing aging information using vertebrae, such as bomb radiocarbon, EPMA, SXFM, and Fukushima radionuclide markers, and encourage members to conduct such studies collaboratively.
3. Continue tagging studies to examine growth, mortality, distribution, and habitat of blue and shortfin mako sharks.
4. Encourage members who have been involved in the cross-reading analyses to prepare the results for publication. The studies provide valuable new information on how to compare growth studies that use different methods.
5. Encourage publication of the meta-analysis approach.
6. Continue research to reduce the uncertainty surrounding life history parameters, such as reproductive cycle and the periodicity of bp deposition.

9. Other matters

The plans for publication and the treatment of documents submitted to this workshop were discussed. Taiwan is planning to publish the updated shortfin mako growth study as an update to the prior work of Hsu (2003) and Chang and Liu (2009). Mexico plans to publish the collaborative cross-reading study conducted with Japan. Japan plans to publish updated shortfin mako growth studies, including the vertebral growth data, size frequency analysis, and chemical analysis.

Regarding the disclosure of the documents submitted to this workshop, the SHARKWG Acting Chair asked that each document be provided for the November data preparatory meeting; at that time, each author will be asked if they permit the posting of the document on the ISC webpage.

10. Review of draft report

The draft report was reviewed and all content provisionally accepted. The SHARKWG Acting Chair will clean up the document and make minor unsubstantial changes. They will then circulate a draft to members for final review.

11. Adjournment

Mikihiko Kai thanked everyone for their participation and contributions to a very productive meeting. He indicated that he looks forward to continual collaborations among national scientists to improve the aging study. A draft of the workshop report will be circulated shortly for review; after it is finalized, it will be included as an attachment to the November SHARK WG meeting.

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Attachment 2. Agenda

SHARK AGE AND GROWTH WORKSHOP

THE INTERNATIONAL SCIENTIFIC COMMITTEE FOR TUNA AND TUNA-LIKE SPECIES IN THE NORTH PACIFIC

19-24 October, 2017
National Research Institute of Far Seas Fisheries
Shizuoka, Japan

1. Opening of the meeting
   1-1. Welcoming Remarks
   1-2. Introductions
   1-3. Meeting arrangements (Adoption of the agenda and assignment of rapporteurs)

2. Overview of meeting objectives and confirmation of goals

3. Review of ongoing work (Cross-reading among national scientists)

4. New information on the aging of shortfin mako, with regional updates

5. Estimation of age from number of growth band pairs (difference of band pair periodicity)

6. Other life history parameters for stock assessment

7. Discussion on the Meta-analysis (candidate growth curves to be used)

8. Work-plan

9. Other matters

10. Review of draft report

11. Adjournment

Attachment 3: List of Papers and Presentations

José Alberto Rodríguez-Madrigal (Mexico)
Standardization of mako shark aging through different vertebrae enhancement methods and comparison of growth estimations from Eastern and Western North Pacific Ocean. Working paper.

José Alberto Rodríguez-Madrigal (Mexico)
Growth estimation update of shortfin mako shark in the Mexican Pacific Ocean, through multi-model inference and different methods for age determination. Presentation and working paper.

Jose Alberto Rodríguez-Madrigal (Mexico)
Tentative birth size of shortfin mako suggested by length compositions collected from Mexican fishery. Presentation.

Julianne Taylor (US)
Cross-readings for shortfin mako shark reference vertebrae among the US, Japan, and Mexico. Presentation.

Kwang-Ming Liu and Shan-Hui Su (Taiwan)
Age and growth estimates of the shortfin mako shark, *Isurus oxyrinchus*, in the Northwest Pacific. Presentation.

Malcolm Francis (New Zealand)
Size, maturity and age composition of mako sharks observed in New Zealand tuna longline fisheries. Presentation.

Mikihiko Kai (Japan)
Estimation of growth from length composition of juvenile shortfin mako, *Isurus oxyrinchus*, in the western and central North Pacific Ocean. Presentation.

Mikihiko Kai (Japan)
Biological parameters necessary to the stock assessment of shortfin mako in North Pacific. Presentation.

Norio Takahashi (Japan)
A potential method for meta-analysis of growth curve for shortfin mako shark in the North Pacific. Presentation.

Suzanne Kohin (US).
Prior ISC SHARKWG aging efforts on shark age and growth. Presentation.

Yasuko Semba (Japan)
General introduction of growth of North Pacific shortfin mako (*Isurus oxyrinchus*). Presentation.

Yasuko Semba (Japan) presentation and working paper:
Update of growth of juvenile shortfin mako (*Isurus oxyrinchus*) in the western and central North Pacific Ocean. Presentation and working paper.

Yasuko Semba (Japan) presentation and working paper:
Application of EPMA to juvenile shortfin mako collected in the western and central north Pacific Ocean. Presentation.