Annex 5

PACIFIC BLUEFIN TUNA AND ALBACORE TUNA AGEING WORKSHOP

International Scientific Committee for Tuna and Tuna-Like Species in the North Pacific Ocean

13-16 November 2013
Shimizu, Japan

1.0 INTRODUCTION

Estimates of population abundance, recruitment, and biomass are highly sensitive to growth curve parameters used in the assessment models. Although growth curves for Pacific bluefin tuna (PBF – *Thunnus orientalis*) and North Pacific albacore (NPALB – *T. alalunga*) have been improving year by year, uncertainty related to age determination is an ongoing challenge for assessing these species. In November 2013, the International Scientific Committee for Tuna and Tuna-like Species in the North Pacific Ocean (ISC) convened the Pacific Bluefin Tuna and North Pacific Albacore Tuna Age Determination Workshop sponsored by the Fisheries Research Agency of Japan. The goals of the workshop were to share information on age determination techniques for NPALB and PBF and to develop standardized protocols for aging to improve the reliability of growth curves used in stock assessments for both species. The meeting was held at the National Research Institute of Far Seas Fisheries (NRIFSF) in Shimizu, Japan.

Dr. Hitoshi Honda, Director of Project Management at NRIFSF, welcomed about 39 participants (see Attachment 1) from Canada, Japan, Spain, Australia, the United States of America, Chinese Taipei, and the Inter-American Tropical Tuna Commission and wished participants a productive meeting.

The meeting was chaired by Osamu Abe (NRIFSF, Japan) with assistance from John Holmes (Fisheries and Oceans Canada). A draft agenda combining presentations and hands-on demonstrations was circulated prior to the meeting and adopted for the meeting (Attachment 2). Sarah Shoffler (National Marine Fisheries Service, USA) was appointed the lead rapporteur for the workshop.

2.0 OBJECTIVES

Several objectives were defined to achieve the goals of the workshop, including:

- Identifying age determination issues for PBF and NPALB,
- Discussing and sharing practical aging techniques among specialists to address age determination issues;

- Developing standardized protocols for tuna aging where appropriate,
- Documenting techniques and protocols in age determination manuals for PBF and NPALB; and
- Discussing the establishment of an Age Structure Exchange procedure between agencies to promote for QA/QC and inter-laboratory calibration of age results.

3.0 PRESENTATIONS

3.1 Ageing Laboratories

Fisheries Research Agency of Japan (NRIFSF & SNFRI) (I. Yamasaki)
FRA has two institutes conducting direct aging of tuna species, National Research Institute of Far Seas Fisheries (NRIFSF) and Seikai National Fisheries Research Institute (SNFRI). The annual ageing work has been done for several tuna species in both institutes and Pacific bluefin tuna is currently a main focus. Sagittal otoliths are used for annual ageing. Both institutes have specialists for otolith reading and several staff for sample preparation, so that around 300 individual fish are aged annually. The reading is compared and cross-checked between readers. As a complement, vertebrae have used for age determination, especially for younger PBF (less than 10 years old). Although the institutes are not currently ageing vertebrate, sampling continues. Otolith bomb radiocarbon analysis has used to validate age estimates by using hardparts. Daily increment analysis is also examined in NRIFSF by four staff. And scanning electron microscopy is used for daily age analysis.

Commonwealth Scientific and Industrial Research Organization (*CSIRO) (J. Farley)*
In Australia, direct ageing of tunas is predominantly undertaken in laboratories at CSIRO's Division of Marine and Atmospheric Research in Hobart and at 'Fish Ageing Services' (FAS) in Portarlington, Victoria. CSIRO's primary role is to collect and archive tuna hardparts and to develop validated methods to estimate annual age. The main tunas species aged are southern bluefin tuna (SBT), South Pacific albacore and Pacific bigeye. CSIRO has two trained tuna ageing specialists who focus on estimating age using transverse sections of sagittal otoliths, although spines, vertebrae and scales are also examined. FAS have 5 staff who provide approximately 12,000-15,000 age estimates annually for 16 fish species under contract to CSIRO. The technique to estimate the age of SBT, developed by CSIRO, was transferred to FAS in 1998. Since that time, the same otolith reader at FAS has determined the age of SBT on a routine basis (~600 SBT otoliths annually). Direct ageing (both annual and daily) of other tunas is undertaken by FAS as required for specific projects. Precision and bias of age estimates is compared between readers at CSIRO and FAS on an annual basis.

Instituto Español de Oceangrafía (IEO) (J.L. Cort)
The IEO in Santander has an equipped laboratory to carry out growth studies of large pelagic species such as Atlantic bluefin tuna (*Thunnus thynnus*), albacore (*Thunnus alalunga*) and swordfish (*Xiphias gladius*). Two researchers and three assistants are currently working, mainly using spines. About 1200 spines are annually interpreted for age determination, and the results are presented to ICCAT species groups. In the case of

bluefin tuna, other objectives are to compare ages estimates from different calcified structures (spines and otoliths) coming from the same specimen.

Inter-American Tropical Tuna Commission (IATTC)(K. Schafer)
The Inter-American Tropical Tuna Commission (IATTC) has a laboratory at their headquarters in La Jolla, California, and suitable equipment for conducting studies on the age and growth of tunas based on evaluations of increments deposited in various hard parts. Currently two staff members conduct tuna age and growth research studies when necessary, in addition to other research responsibilities as requested by the Commission. Routine production ageing of tunas is not undertaken by IATTC, but specific age and growth studies have been published for skipjack, yellowfin, Pacific, and bigeye tunas.

Southwest Fishery Science Center (SWFSC) (S.M. Shoffler)
The NOAA Southwest Fisheries Science Center (SWFSC) laboratory in La Jolla, California annually collects sagittal otoliths from a number of fisheries and species. For large pelagics, about 50-100 North Pacific albacore otoliths are collected from the surface fishery; about 100 sagittal otoliths from various species are collected from the California sport fishery and about 20 sagittal otoliths are collected from the CA drift gillnet fishery. In addition, the SWFSC has collected about 30 North Pacific albacore and a few Pacific bluefin tuna sagittal otoliths during a 2013 research cruise. The SWFSC currently does not conduct production ageing on tunas. In addition, the SWFSC has collected some vertebrae from mako, blue and thresher shark. These are regularly used in oxy-tetracycline tagging and age-validation. For small pelagics, the SWFSC collects 50-200 Pacific sardine and 500+ Pacific mackerel sagittal otoliths each year from research cruises in the California Current. The SWFSC has one otolith ager who reads these otoliths whole in distilled water. In addition, the SWFSC has collected market squid statoliths from various research cruises over the years. One ager reads these statoliths bonded to slides. SWFSC agers use Leica, Olympus and Nikon dissecting and compound microscopes as well as a SPOT mosaic color camera and a SPOT RTS slider imaging system.

Texas A&M University (D. Wells)
An overview of the age and growth work was provided along with a short introduction on our new fisheries research lab at Texas A&M University. A total of five graduate students and two technicians are currently working in the lab at this moment. Current age and growth studies are ongoing for several shark species including Shortfin Mako (*Isurus oxyrinchus*), Blue (*Prionace glauca*), and Common Thresher (*Alopias vulpinus*) Sharks. Less than 100 individuals combined across all three species are being aged using vertebral aging techniques including both light microscopy and x-ray methods for age validation studies. North Pacific albacore and Pacific bluefin tunas are sometimes aged for the otolith chemistry work. A recent study on albacore involved annual ageing of 485 individuals and another on daily aging of albacore used 128 individuals. Currently, no routine aging is occurring for Pacific tuna species; however, each individual is provided an age during the sectioning process for movement studies utilizing otolith chemistry techniques.

Fisheries and Oceans Canada (D. Gillespie)

The Sclerochronology Lab of Fisheries and Oceans Canada is located at the Pacific Biological Station in Nanaimo and has nine personnel, consisting of two biologists, six fish ageing technicians, and a database technician. The lab ages approximately 120,000 fish annually, comprising about 15,000 groundfish of various species of rockfish, *Sebastes* spp., sablefish (*Anoplopoma fimbria*), hake (*Merluccius productus*), Pacific cod (*Gadus macrocephalus*), lingcod (*Ophiodon elongatus*), and flatfishes, gadids, 75,000 Pacific salmon (*Oncorhynchus* spp.), 25,000 Pacific herring (*Clupea pallasii*) and sardine (*Sardinops sagax*) and 5,000 shellfish, largely geoducks (*Panopea generosa*) and Manila Clams (*Venerupis philippinarum*). Otoliths, scales, fin sections and shell parts are the most common structures used to age finfish and shellfish. The SCL uses Leica Mr Z7.5 stereomicroscopes with an Intralux fiber optic reflected light source and transmitted light base, Leica DM2000 compound microscopes and two Leica DFC295 3 megapixel cameras. All species are aged using a single ager system with 10-20% of each sample independently re-aged by a second reader as part of the QA/QC protocol. Readings are compared and discrepancies resolved by the readers. Age class is assigned based on the internationally accepted January 1st birthdate, taking into account the catch date of the fish. The SCL has a rigorous training process for novice readers that prepares them to fully contribute to production aging of all species after 2-3 years. The lab oversees a large archival collection of ageing structures that are used by other research studies such as DNA analysis, micro-elemental, isotope and bomb radiocarbon studies. Approximately 90% of the available working hours are focused on production aging for stock assessment purposes, with the remaining 10% devoted to research. Some ongoing research projects include the testing of new methods for otolith preparation, preparing ageing manuals to document procedures for various species, and experimenting with a direct input system for all age data using tablet computers at all microscope workstations.

National Taiwan University (J.J-C. Shiao)

The Fish Ecophysiology lab in the Institute of Oceanography, National Taiwan University (IONTU) is managed by Associate Professor Jen-Chieh, Shiao. The lab is equipped with embedding silicon molds, la ow-speed saw (Isomet, Buehler, Evanston, IL, USA), a grinder-polisher machine (Buehler, Metaserv 2000, Evanston, IL, USA), a stereo microscope (Olympus SZ12, Japan) and a compound microscope (Olympus BX-51, Japan) equipped with a digital camera (DP-71, Olympus, Japan) to prepare samples for examination of otolith structure and age determination of individual fish. IONTU also has micromill (NewWave, ESI Corporation, USA) and isotopic ratio mass spectrometry (Finnigan MAT 253, Thermo Electron Corporation, Germany) connected to carbonate inlet system (Kiel carbonate device IV, Kiel, Germany) that can be used to measure stable isotope composition of otoliths. Approximately 170 PBF otoliths (2011-2013) and 100-300 SBT otoliths (since 2003-2013) are processed and aged annually. Each sample is read 3 times by the same person and the average percent error (APE) is calculated and used for quality control. When the reading results are not consistent among the three readings, a second reader will read the samples and address discrepancies through discussion among both readers to produce a final resolved age. The criteria used to identify and read the annuli are mainly those developed for southern bluefin tuna as described in CCSBT

(2002) and Pacific bluefin tuna Shimose *et al*. (2009).

Pacific bluefin tuna (PBT) otolith research at Tokyo University of Marine Science and Technology (TUMSAT) (C.A. Strüssmann)
The Laboratory of Population Biology at TUMSAT works on the development of otolith-based methods to identify the natal origin of young-of-the-year PBF landed in Japan. This research is carried out under contract for the Fisheries Research Agency of Japan and is tied to undergraduate and graduate students' theses work. Staff includes the PI (Carlos Augusto Strüssmann, Professor) and a variable number of students each year. We integrate information on otolith core region microchemistry (elemental composition obtained by Electron Probe Microanalysis), birthdate (calculated from catch date and estimated age in days), lifetime growth rate (calculated from the size at catch and age in days), and larval growth rate (estimated from daily increment width measurements) in the analysis. Otolith specimens are prepared as epoxy-embedded, thin (less than 100 µm), nearly-transversal sections that include the otolith primordium, and are mounted on glass slides. We are able to process/analyze up to 200 specimens/student/year. Our equipment includes a low-speed saw, semi-automated grinding (for coarse, wet work) and polishing (for use with diamond/alumina) machines, microscopes (normal and compound, transmitted, reflected and fluorescent light sources), RATOC otolith daily ring measurement system, Image-Pro Image Processing Software, Hitachi SEM (field emission), FEI Quanta ESEM (variable/environmental pressure), JEOL five-channel EPMA (electron probe microanalyzer), New-Wave micromill, and Thermo Scientific ICP-MS with laser-ablation. An additional objective of our research on PBF is to develop simple, reliable methods for otolith specimen preparation that may be suitable for mass, semi-automated daily/yearly age determination.

3.2 Case studies

Age estimation and validation for southern bluefin tuna (J. Farley)
This presentation summarized research undertaken since the mid-1990s to develop a validated method to estimate the annual age of southern bluefin tuna (SBT). Initially, estimates of daily age from counts of microincrements confirmed that 45-55 cm fork length (FL) fish are 1 year old, and 70-80 cm FL fish are 2 years old. Scales, vertebrae and sagittal otoliths along with several preparation and sectioning techniques were subsequently examined and it was found that transverse sections of otoliths produced the most accurate annual age estimates for SBT. The age estimation method was validated through a combination of marginal increment analysis, a strontium chloride mark-recapture experiment, and a bomb radiocarbon study. Several aspects of age determination were summarized including otolith interpretation criteria, the importance of trained readers applying the interpretation criteria consistently, estimating precision and bias, and the advantages and disadvantages of using serial transverse sections of otoliths. An annulus consists of a wide opaque zone and a narrow translucent zone under transmitted light. The wide opaque zones form during summer and fall (assumed fast growth) while the narrow translucent zones appear to form during the winter months (assumed slow growth). The opaque zones are used to estimate the age of fish, but are

only counted if a translucent zone is present after the opaque zone. The age determination methods developed at CSIRO were transferred to scientists at CCSBT member countries via the "Direct Age Estimation Workshop" held in Australia in 2002. Finally, an algorithm to calculate decimal age of SBT was presented (based on an assumed birth date of January 1, the known capture date, and the opaque zone count) and the procedures for routine age estimation of SBT in Australia was summarized.

Age estimation and validation for south Pacific albacore (J. Farley)
This presentation described methods to estimate the annual age of South Pacific albacore from counts of opaque growth zones in transverse sectioned otoliths collected across the south-west Pacific Ocean. Initially, counts of daily increments (microincrements) in sectioned otoliths confirmed that 1 year old fish are approximately 50 cm FL, and that the first annual opaque zone forms before the first birthday (365 days). The annual periodicity of opaque zones in otoliths demonstrating that one opaque and one translucent zone are produced in a year, was confirmed through marginal increment analysis and an oxytetracycline mark–recapture experiment. Based on the results of these validation methods, it was shown that opaque zones form over the summer and are completed by autumn to winter. The protocol used to estimate the age of SBT is also used for South Pacific albacore: opaque zones are only counted if a distal translucent zone is clearly visible closer to the edge. When counts from otoliths and sectioned dorsal spines of the same fish were compared, it was concluded that the ray counts were bias relative to the otolith counts possibly due to poor clarity and vascularisation of the core in many spines. The presentation highlighted the importance of recording either the marginal increment ratio or the edge type when counting opaque zones in otoliths. Decimal age can then be calculated which accounts for the relative state of completion of the increment on the distal edge of the otolith.

Methods of analysis for ageing eastern and western Atlantic bluefin tuna (Thunnus thynnus) (J.L. Cort)
This presentation was a historic review of the Atlantic bluefin tuna (*Thunnus thynnus*) (ABFT) growth studies carried out in the Centre of Santander of the Instituto Español de Oceanografía (IEO). Spines of the first dorsal fin of ABFT have been collected, prepared and read since 1979. Samples are collected in landing ports of the eastern Atlantic and Mediterranean fisheries. Depending on the size of the samples, precision rotating diamond saws are used for sectioning. If fish are between 50-140 cm (FL), a low speed saw is used (IsoMet, Buehler); if fish are > 140 cm (FL), a linear precision saw is used (IsoMet 5000, Buehler). At present, more than 10,000 spines have been prepared and read in the laboratory since 1979. In recent years, otoliths and spines have been collected simultaneously from 200 fish annually. Spines are read using transmitted light and age is assigned assuming that a translucent band is followed by an opaque band and these bands are formed annually. When the central part of the spine is reabsorbed, the location of the first inner visible translucent band is based on the mean diameter of this band, which has been previously estimated. The longest arm of the sectioned sagittae otolith is used to interpret age. Age estimates between readers for spines and otoliths are compared using ageing precision indices such as average per cent error, coefficient of variation and the weighted mean percentage agreement. About 600 albacore (*Thunnus alalunga*) and 200

swordfish (*Xiphias gladius*) spines are also collected, prepared and read annually. The ageing team consists of two scientists and three assistants.

An overview of published investigations on age and growth of yellowfin, bluefin, and bigeye tunas in the eastern Pacific Ocean, by staff of the Inter-American Tropical Tuna Commission (K. Schaefer)
Validation studies conducted using oxytetracycline marking in conjunction with tag recapture experiments for skipjack, yellowfin, bluefin, and bigeye tunas demonstrated daily microincrement deposition in sagittal otoliths. The age and growth of yellowfin tuna was described from a sample of 196 fish, 30-170 cm FL, collected in 1977 through 1979 from purse seiners fishing north of the equator and east of 137°W. The number of increments on a sagittal otolith of each fish was used as a direct estimate of its age in days. Age estimates of bluefin tuna were made from daily microincrement counts in sagittal otoliths and annual increments in vertebral centra up to about 5 years of age. Age estimates of bigeye tuna were made from daily microincrement counts in sagittal otoliths for fish up to about 4 years of age. The growth rate derived from tagging data was compared estimates based on length-at-age data, and the two independent sets of data show similar decreases in growth with increasing length. A modified "Laslett-Polacheck-Eveson (LEP)" statistical approach (fitting Gausian mixtures to length-frequency data, extracting summary statistics and fitting parametric growth models such as the von Bertalanffy growth model, to the summary statistics) was developed so as to integrate age-at-length and tagging length-increment data for bigeye tuna from the eastern Pacific Ocean. Integrating the two sources of data into a single-step modeling approach is ideal, as the age data from counts of daily increments on otoliths is only available for fish younger than four years old, and the tag-recapture data come from a mixture of young fish and an older fish up to about 10 years of age. Several improvements resulted from integrating the two complementary data sets.

3.3 Current status and issues of age determination techniques

History, current status, and issues of age and growth studies of Pacific bluefin tuna (T. Shimose, SNFRI)
The history of age and growth studies on Pacific bluefin tuna (PBF) were reviewed, and the current age determination method and growth curve (Shimose *et al*. 2009) were introduced. Three issues were posed for the workshop to consider. The first issue is the possible miscount of annual rings. Periodicity of opaque zone formation was validated by edge type analysis only for older (ca. >10^{th}) annuli in larger individuals but not for earlier annuli in smaller individuals. There are some outliers in the age-length relationship, consistent with the inclusion of overestimated and underestimated ages in the growth curve calculation. The second issue is the weak repeatability of counting by a single reader. Annual increments were counted by one reader with low precision (CV = 6.38). The repeatability of the counts and precision can be improved with the addition of a reader and an ageing manual to guide readers. The third issue is that the predicted fork length from the estimated growth curve was sometimes inconsistent with the length frequency distribution of the catch, especially for the younger ages. This may be

improved by combining the two sources into single growth model.

Current status of age determination of North Pacific albacore tuna (D. Wells)
The presentation focused on the age and growth paper recently published by Wells et al. (2013). Accurate estimates of age and growth parameters are important for fisheries management because these values affect stock assessment models. Age and growth of North Pacific albacore (*Thunnus alalunga*) was assessed by examining annual growth increments in sagittal otoliths from 486 fish collected in different regions of the North Pacific Ocean. A wide size range of albacore (52-128 cm fork length, FL) was collected in an attempt to incorporate size-at-age information over juvenile and adult life history stages. Overall, ages ranged from 1 to 15 years, with the majority of fish between 2 to 4 years of age. Growth models were fitted to otolith-based size-at-age well, and based on Akaike's Information Criterion (AIC) corrected for small sample size, the specialized von Bertalanffy (VB) model provided the best fit to the otolith data. The estimated biological parameters of the specialized VB model were L_∞=124.1 cm FL, K=0.164 year^{-1}, and t_0=-2.239. Daily microincrements were counted to verify correct age-1 assignments for fish 55 to 61 cm FL, whose daily ages ranged from 378 to 505 days. In addition, dorsal fin spines and length frequency (LF) analysis were used to obtain estimates of size-at-age and to corroborate results from otolith-based techniques. Modeling exercises resulted in nearly an order of magnitude difference in spawning stock biomass (SSB) when comparing growth parameters obtained from this study to previous stock assessment models of North Pacific albacore. Results suggest North Pacific albacore is a relatively long-lived tuna species and provide updated biological parameters useful for future stock assessment models.

3.4 Technical issues with age determination

Interpreting initial annual rings for Pacific bluefin tuna (T. Ishihara)

By using the fork length data collected by RJB (Research project on Pacific bluefin tuna), body size transition could be followed until juveniles reached 100 cm in FL, at about 2 years old. Thus, within this range, the age could be estimated by its FL. By using the otolith from age-0, age-1, and age-2 fish estimated by its FL, the 1st and 2nd (if exist) annulus locations were noted and marginal increments were analyzed for 219 samples from fish ranging 50 to 100 cm in size. On the cross section of otoliths, morphological characteristics appeared as opaque zones along the outer edge of the ventral arm, and opaque lines on sulcus side of the ventral arm. In addition, there were textural changes, crowded think lines, on the proximal side of the 1st opaque zone. The distance from the primordium to the 1st opaque zone were measured and found that this structure often appeared from 0.5 to 1.0 mm from the primordium of the otolith. The distance between the 1st/2nd opaque zones and the edge of sulcus side of ventral arm was measured for marginal increment analysis. The marginal increment ratio (MIR) of the 1st opaque zone was calculated and the monthly transition of MIR indicated that 1st opaque zone was formed once in a year, between December and June. As a conclusion, 1st opaque zone appeared between 0.5 - 1.0 mm from the primordium and characterized by the following features is considered as initial annulus.

- ✓ Opaque zone in outer edge of Ventral arm
- ✓ Opaque lines in Sulcus side of Ventral arm
- ✓ Crowded thick lines appeared proximal side of the opaque zone

Technical issues for age determination of North Pacific Albacore tuna (D. Wells)
Four key technical issues parameters and recommendations were identified in for ageing North Pacific albacore. 1. Interpreting the band pairs. First using a training set of otoliths (when available) and training the reader to look for the proper band pairs in the species being studied is recommended. In the case of albacore, readers are looking for dark smudges representing the opaque band. 2. Interpreting the initial band. This is accomplished by referencing the first inflection point and using distances from validated daily age estimates. 3. Following the bands across the cross-section is often difficult and not always reliable. Other methods include identifying small bulges along the ventral edge of the otolith. 4. Focus on the macro-structure (not microstructural increments) when examining otoliths to estimate annual ages. A series of images were examined to visually discuss the process of estimating ages for a suite of albacore samples.

Albacore age comparison and methods (D. Gillespie)
An albacore otolith exchange was conducted between the Sclerochronology Lab(SCL), Nanaimo, Canada and the SWFSC, La Jolla, California (Contact: Dr. David Wells, Texas A&M University). The SCL had previously aged a small number of albacore and looked at this exchange as a training opportunity in preparation for this workshop. The two labs independently aged 50 saggital otoliths and compared results after the ageing had been completed. A combination of preparation methods were used by the SCL: transverse thin sections, as well as burnt and baked transverse cross-sections. Preparation methods were reviewed and examples of otolith growth patterns age comparisons were discussed. The results of the age comparison showed that the CAN ages were younger overall than the US ages and that this bias was greatest for the older fish in the sample. Breaking down the results by preparation method showed that there was less variation in age with the transverse thin section preparations than with the burnt and baked methods. A summary of the advantages and disadvantages of each preparation method for albacore otoliths was presented and recommendations for age criteria and protocols to include in the manual were suggested.

Age structure of Pacific bluefin tuna landed in Taiwan and preliminary investigation of the population mixing inferred by stable isotopic analysis (J.J-C. Shiao)
Size and age composition data of PBF landed in Taiwan during the past 20 years were reviewed. The fork length of the PBF landed in Taiwan ranged from 170 to 260 cm during 1993-2013. The size of the majority of PBF landed in Taiwan has increased from approximately 220 cm before 2008 to approximately 230 cm in 2009 and 2010 and continued to increase to approximately 240 cm in 2013. The age of the majority of PBF landed in Taiwan increased from 12 years before 2008, to 13 years in 2009, to 14-16 years in 2011 and to a range of 13-19 years in 2013. Some possible reasons, including a change in fishing grounds and depletion of young PBF leading to in the size/age range of PBF available to the fishing fleet and landed in Taiwan, were discussed. More fishery

data collected from the same period, especially from the Nansei Islands, may provide useful comparisons and broaden perspectives and help explain change in the PBF size/age composition in Taiwan. So far, there is no robust explanation of the increase in the size and age of PBF landed in Taiwan. Stable isotopic analysis to discriminate the natal origins of PBF was demonstrated. The first peak of $\delta^{18}O$ (winter signal) in the otolith appears less than 1000 µm from the core in otoliths of PBF hatched in the Japan Sea and around 1200-1600 µm from the otolith core of PBF hatched in the Pacific Ocean. This disparity in $\delta^{18}O$ peak locations provides useful information to determine the natal origin of PBF of all ages. Based on preliminary data age 1+ PBF with different natal origins are mixed to varying degrees in the Japan Sea, Tsushima Strait, and southern Japan. Most PBF spawning in the western Pacific Ocean were estimated to be hatched in this area, while some estimated hatched in the Japan Sea. More analyses are required to provide sufficient data that can be used to estimate the mixing rate of the PBF collected at different fishing grounds.

Age determination of Pacific bluefin tuna (Thunnus orientalis) by reading rhythmic bands on vertebrae (A. Nagata)
The Pacific Bluefin Tuna Biology Group of NRIFSF is examining ageing using calcified structures other than otoliths. Vertebrae show clear annual bands in young ages (less than 10 years old), which is a difficult period to identify annuli and age PBF using otoliths, although annuli on the vertebrae gets unclear with increasing age. For over 300 fish, age was examined by two methods; by using 35^{th} vertebrae and otolith , and the results were compared. The growth curve based on vertebrae ageing appeared to be close to that of otolith ageing, while over age-10 disparity between ages counting by two techniques became wider. This result indicates that vertebrae could be compensatory materials for ageing when annuli of otolith are very unclear under age-10. In the future, new agers will be added and trained, and the vertebrae will be observed directly rather than images of the vertebrae.

Validation of annual rings using radioisotope for Pacific bluefin tuna (T. Ishihara)
Bomb radiocarbon dating was conducted to validate otolith age estimates in recently calcified otoliths from 0+ fish and fish born after the 1980s. The $\Delta^{14}C$ from otolith cores in older fish was measured and the estimated ages were compared with ages estimated from otolith increments. First, we measured the $\Delta^{14}C$ in whole otolith of age-0+ and age-1+ fish. Because the calcification year of these materials are obvious, we used these values as controls. The $\Delta^{14}C$ trends of the above samples are consistent with the historical trends for the dissolved inorganic carbon in the sea water and in corals reported in the previous studies. Next, using fish ranging 4 - 22 estimated years-old, we measured the $\Delta^{14}C$ around the primordium of otolith which was calcified atage-0/age-1. The $\Delta^{14}C$ values around the primordium in the otolith at the estimated birth year were close to the control values. The birth years estimated using $\Delta^{14}C$ and that estimated using otolith annuli are consistent. Therefore we concluded that the ages estimated by otolith annuli have some reliability.

Microstructure change of age-0 fish otoliths (I. Yamasaki)
Metabolic change, which relates to water temperature, is possibly the main factor

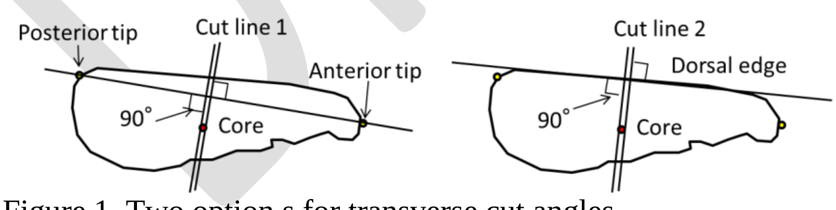
influencing the formation of annual patterns on hard tissues during the immature period of PBF. Marginal increment analyses confirmed that the first annual ring on PBF otoliths forms during winter. Our research found a structure with quite narrow intervals of microincrements on the oblique section of age-0 PBF otoliths. In our study, 218 individuals from 20-60cm FL were analyzed, and the microincrements were counted to estimate the period of this structure. The structure was observed only in large individuals (over 58 cm in FL), beginning at an age of 160 days on average. The estimated period of this structure was matched to the season with low water temperature for most individuals. Therefore, this particular structure may relate to the low water temperature of winter.

4.0 RECOMMENDATIONS

Otolith section preparations

- Transverse thin otolith sections are commonly used to age both albacore tuna and SBT; ALB scales and vertebrae were explored in the past, but success was not high. Transverse thin sections are also used to age PBF, but there was evidence presented at this workshop that vertebrae could be helpful, especially for estimating ages of fish younger than 10 yrs of age.
- The otolith core should be marked lightly with pencil so that it can be easily located for sectioning.
- It is recommended that serial transverse thin sections are prepared, ensuring that the first section is taken through the otolith primordium in order to establish the location of the inflection point.
- The photos of the otolith cross-sections should include a measurement scale.

Cut Angle

- Transverse sections cut at 90° to the longest axis are recommended for counting annual growth increments and estimating age. Two options of transverse section below are equally recommended (Cut line 1 and 2 are used by Shimose and Wells, respectively).
- 

- Figure 1. Two option s for transverse cut angles
- Transverse sections can also be used to count daily growth increments however, frontal sections running from the primordium through the post-rostrum are better for counting daily growth increments.

Thickness of sections

- Section between 0.3-0.5 mm (especially 0.4 mm) thick are most commonly used for counting yearly growth increments using transmitted light.

- Thin sections are polished to the point where microincrements can be seen along the entire counting path for daily increment counts.

Serial Sections

- Otoliths cut into serial sections and mounted on a single slide reduce the risk associated with relying on a single cut, which may lead to a poor quality sections that affect the overall precision and accuracy of ageing.
- Serial sections provide different views of the increments and options for viewing the primordial slice, which is critical for accurate ageing of most tuna species discussed at this workshop.
- Ensure that at least one section includes the primordium/core because this section will consistently include the first inflection point. This can be used as a measurement key to establish the location of the first opaque zone.
- The preparation of serial sections requires more time, larger slides, and the use of a high-speed saw.

Equipment for reading/interpreting sections

- Dissecting and compound microscopes with transmitted light are used for counting annual growth increments.
- Compound microscopes have proven to be more suitable for counting daily increments in tunas than dissecting microscopes.
- Increments can be counted directly using a dissecting or compound microscope or using photos.
- Photos of otoliths or other hard parts used for aging are an important part of the documentation process and should be compiled, labelled, and stored routinely.
- Photos should show the main counting axis and a size scale; measurement scale marks denoting the location of each annulus that is counted should be added to the image during post processing as a permanent record.

Identifying the position of the first annulus

The position of the first annulus in many tunas is often difficult to determine visually. Many laboratories that routinely age tunas use the estimated average measured distance from the primoridum (core) to the 365th daily increment to establish the location of the first opaque zone (which should occur before the 365th daily increment) when it cannot be clearly determined visually. This procedure is followed when aging South Pacific albacore and SBT (See CCSBT, 2002*[1]).

As the 1st/2nd annuli are sometimes very vague, some guideposts such as average distances for the 1st/2nd annuli from the primordium/1st inflection point could be useful marking the 1st/2nd annuli. Use sections with a clear 1st/2nd opaque zones to establish the average distances from the primordium/1st inflection point as references. The positions of above clear 1st/2nd opaque zones should be verified with daily age analysis. Preliminary study for PBF shows that the distance from the primordium is <1.0 mm and 1.0-1.4 mm for the 1st and 2nd opaque zones, respectively.

Marginal Increments

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[1] CCSBT (2002) A manual for age determination of southern bluefin tuna, *Thunnus maccoyii*.

- Measure the marginal increment ratio in order to determine the relative state of completion of the last opaque zone and to estimate decimal age of the fish.
- Classify and record otolith terminal edge type; whether there is wide or narrow opaque material and whether there is translucent material beyond that "wide" and "narrow" material. These are subjective classifications and as long as readers consistently record this information, the subjectivity will not matter.

Imaging Software

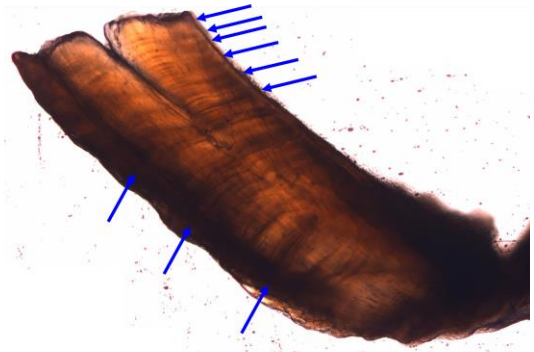
- Use of imaging software is optional. It can be used to:
  - Set a counting axis and initial marks for counting on the image. The initial marks represent approximate locations of annuli. Since these marks are not fixed, they can be moved to different locations after checking the otolith with a microscope for confirmation. These marks can be moved and, if there is uncertainty, to check the otolith on the microscope. When reading is fixed, the photo with fixed marks should be kept for reference.
  - Measure distances between marks.
- The most common commercial off-the-shelf (COTS) software package used for this type of analysis is ImagePro+. Free software includes ImageJ among others.

General recommendations for reading and interpreting sections

- Tilt slides of sectioned otoliths to get clearer views of annuli, especially of what's on the edge.
- Standardize measurement methodology – for example, start from the primordium/core and measure/count increments to the inflection point and then to the terminal edge of the otolith.
- Chose very clear samples to measure the location of the first and second rings and compare them to other samples.

Interpretation of annuli

- Focus on macrostructures when counting annual increments and not the microstructure.
- Under transmitted light, alternating light and dark bands will be visible. It is generally easier to count opaque zones but translucent zones can be counted. Record whichever is counted as this makes a difference in terms of how the edge is interpreted.
- Rings should be counted without reference to information on fish size or catch location.
- Counting generally occurs along the ventral arm of the otolith; the dorsal arm is seldom used for this purpose.
- Agers can switch from one edge (sulcal or distal) of the "arm" in order to follow the bands.
- Opaque zones in Pacific bluefin tuna and North Pacific albacore tuna may not be as clear as those in less migratory species and opaque zones may appear as smudges. Crenations (bumps or bulges) along the edge may indicate locations of the end of the smudges (or increments).
- Crenations on outer edges can suggest bands but readers should not rely on them.

- 
  Figure X. Crenations on outer edge (arrows on the left side)
- Spacing between annuli in earlier/juvenile years is generally wider than spacing between annuli in later years. Use relative spacing to identify checks (false annuli) from actual annuli.
- Document the maximum and minimum counted ages as measures of ageing error.
- Reference photos with detailed description of useful characteristics indicated above (smudges, crenations, bumps or bulges) and check points to distinguish false and true annuli should be prepared for free access on the ISC homepage. It should show different age groups for Pacific bluefin tuna and albacore tuna and the difference between checks false annuli vs. true annuli.
- Use a decimal age system when recording ages.

Utilization of other information

- Standard birthdates for tunas are typically the middle of the spawning period and they can be back calculated using daily increment counts in young fish (preferably 1-2 yr old fish). However a large sample size is required to get an accurate estimate of the birthdate. Therefore, estimating birthdate from reproductive biology is preferred.
- When hard parts are utilized to estimate age, the deposition rate of daily or annual marks throughout the length range should be validated
- Indirect validation with daily ring counts and marginal increment analysis are often used to determine the time of opaque zone formation.

Other Issues

- Novice agers should get a training set of otoliths to start to train eye to identify subtle patterns. An appropriate training scheme should be prepared in each lab to keep reading consistent.

Future Research Considerations Noted by the WS:

- Age determination manual for PBF and NPALB with a new criteria discussed in this WS should be published.
- Reference photos showing detailed of interpretation of annuli for PBF and NPALB should be prepared for free access on the ISC homepage.
- Age-at-length data from hard parts and growth data from tagging studies should be compared and integrated into growth models; e.g. see EPO bigeye tuna, Atlantic bluefin, and South Pacific bluefin tuna assessments.

- The spatial and temporal variability in age at size should be investigated as it can have profound impacts in stock assessments
- Daily ages of Pacific bluefin tuna should be verified. First cut 0.4 mm section to get first annulus then section otolith down in order to then age. OR do 2 pairs of sections and count annulus on the thicker one and daily increments on second smaller section.
- Consider whether imaging software can make a contour of the surface to be used for daily increments for younger fish and verify with “traditional” daily counts.
- Determine the location of first year/annulus in North Pacific albacore tuna – what is the size, measurement, distal indentation, using the surface?
- Validate Pacific bluefin tuna age determination using vertebrae
- Validate the first annual ring of Pacific bluefin tuna with daily annulus counts via the SEM technique (see Yamasaki *et al*. presentation)
- Establish the location of the 365th and 730th daily increment for both Pacific bluefin and North Pacific albacore tunas.

Appendix 1 – List of Participants

Participant	Affiliation	Country	Email
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Attachment 2 – Workshop Agenda

INTERNATIONAL SCIENTIFIC COMMITTEE FOR TUNA AND TUNA-LIKE SPECIES IN THE NORTH PACIFIC OCEAN (ISC)

“Pacific Bluefin and North Pacific Albacore Tuna Age Determination Workshop”

PACIFIC BLUEFIN TUNA WORKING GROUP
ALBACORE WORKING GROUP

13-16 November 2013

National Research Institute of Far Seas Fisheries (NRIFSF),
Fisheries Research Agency (FRA),
Shimizu, Shizuoka, Japan

Day 1 - 13 November (Wed): Current Issues for Age Determination Techniques

0900: Registration

Agenda 1. Opening of the WS

0930: Welcome remarks
Introduction of the participants
Goals of the workshop, including the desire to produce PBF/ALB manuals
Adoption of the agenda
Appointment of chairpersons
Appointment of rapporteurs
Logistical information

Agenda 2. Overviews of ageing labs

1000: FRA (NRIFSF & SNFRI) ... (I. Yamasaki)
CSIRO ... (J. Farley)
ELO ... (J.L. Cort)
IATTC ... (K. Schafer)
Texas A&M Univ ... (D. Wells)
Fisheries & Oceans Canada ... (D. Gillespie)
National Taiwan Univ ... (J.J-C. Shiao)
Tokyo Univ. of Marine Science and Technology ... (C.A. Strüssmann)

Agenda 3. Case Studies

1030: Southern bluefin tuna (title tetative) ... (J. Farley)

1210: ***<Lunch>***

1330: South Pacific albacore (title tetative) ... (J. Farley)
1410: Methods of analysis for ageing eastern and western Atlantic bluefin tune (*Thunnus thynnus*) ... (J.L. Cort)
1450: An overview of published investigations on age and growth of yellowfin, bluefin, and bigeye tunas in the eastern Pacific Ocean, by staff of the Inter-American Tropical Tuna Commission ... (K. Schafer)

1530: *<Coffee Break>*

Agenda 4. Current Status and Issue of Age Determination Technique
1600: PBF ... (T. Shimose)
1630: NPALB ... (D. Wells)

1800-2000: *<Reception>*

<u>Day 2 - 14 November (Thu) - Information Sharing</u>

Agenda 5. Technical Issues for Age Determination
0900: Interpreting initial annual rings for PBF ... (T. Ishihara)
0930: Interpreting initial annual rings for ALB ... (D. Wells)
1000: Albacore Age comparison and methods ... (D. Gillespie)
1030: Age structure of PBF landed in Taiwan and preliminary investigation of the population mixing inferred by stable isotopic analysis ... (J.J-C. Shiao)
1100: Age determination of PBF using Vertebrae ... (A. Nagata)
1130: Validation of annual rings using radioisotope for PBF ... (T. Ishihara)
1200: Daily age determination of PBF using SEM... (I. Yamasaki)
1230: ***<Lunch>***

Agenda 6. Hands-on demonstration on reading annual/daily rings
<chairperson: John Holmes, Osamu Abe>

Discussion - how to interpret the banding patterns (including discussion of images shared prior to workshop)

<u>Day 3 - 15 November (Fri) - Hands-on Demonstration</u>

Agenda 6. Hands-on demonstration on reading annual/daily rings <cont.>
<chairperson: John Holmes, Osamu Abe>
Hands-on demonstration - how to take photos using the microscope
Discussion - how to prepare the otolith samples

1200: <Lunch>

Discussion – appropriate age structures
Discussion – International birthdate and standardized terminology
Discussion – Inter-laboratory calibration

Day 4 - 16 November (Sat) - Discussions and Reporting

Agenda 7. Elaboration of Age Determination Manual
0900: Consensus on protocols for age determination
Examine aging manuals for PBF and NP-ALB.

Agenda 8. Recommendations and manual preparation schedule
1600: Recommendations
Manual preparation schedule

Agenda 9. Closing
1700: Closing remarks

***Hands on demonstration**
Participants are requested to bring their own otolith samples for age determination.

1. Using microscope and otolith samples, participants are requested to demonstrate how to take photos.
2. Viewing several otolith photos on the screen, discuss how to interpret the banding patterns.