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REPORT OF THE INTERNATIONAL WORKSHOP ON THE AGEING OF YELLOWFIN AND BIGEYE TUNA

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SUMMARY

Given the number of laboratories now ageing bigeye and yellowfin tunas using 'annual ageing', it was important to hold a workshop to ensure consistency across laboratories. Otolith preparation and ageing protocols were found to be in general agreement. Preliminary results from recent oxytetracycline mark-recapture studies suggest that daily increment counts may only be useful to age very young fish, while annual increments appear to be deposited on an annual basis throughout life. A similar pattern was previously found in a strontium-chloride mark-recapture study of bigeye tuna. Maximum age estimates of 16–18 years for yellowfin and bigeye have been validated with bomb ^{14}C dating. The group agreed to a standardized protocol for assigning otolith edge type and that only fully formed opaque zones would be counted as annuli. Ageing algorithms for estimating fractional age were presented. Participants concluded that the current annual age estimation protocols are valid, and that ageing both species using counts of annual growth increments is possible and recommended to ensure that the oldest individuals are not being systematically underaged and to capture the variation in length-at-age.

RÉSUMÉ

Étant donné le nombre de laboratoires qui déterminent maintenant l'âge du thon obèse et de l'albacore en utilisant la "détermination annuelle de l'âge", il était important d'organiser un atelier pour assurer la cohérence entre les laboratoires. Les protocoles de préparation et de détermination de l'âge des otolithes se sont avérés globalement cohérents. Les résultats préliminaires de récentes études de marquage-récupération avec oxytétracycline suggèrent que les comptages d'incréments quotidiens pourraient n'être utiles que pour déterminer l'âge des très jeunes poissons, alors que les incréments annuels semblent se déposer sur une base annuelle tout au long de la vie. Un schéma similaire avait déjà été trouvé dans une étude de marquage-récupération avec du chlorure de strontium sur le thon obèse. Les estimations de l'âge maximum de 16-18 ans pour l'albacore et le thon obèse ont été validées par la datation à l'aide de carbone radioactif 14C. Le groupe s'est mis d'accord sur un protocole standardisé pour l'attribution du type de bord de l'otolithe et sur le fait que seules les zones opaques entièrement formées seraient comptées comme des anneaux. Des algorithmes de détermination de l'âge pour l'estimation de l'âge fractionnaire ont été présentés. Les participants ont conclu que les protocoles actuels d'estimation annuelle de l'âge sont valables et qu'il est possible de déterminer l'âge des deux espèces en utilisant des comptages d'incréments de croissance annuels. Ils ont recommandé de veiller à ce que l'âge des spécimens les plus âgés ne soient pas systématiquement sous-estimé et de saisir la variation de la longueur à l'âge.

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RESUMEN

Dado el número de laboratorios que están determinando la edad de patudos y rabiles usando la «determinación de la edad anual», era importante celebrar un taller para garantizar la coherencia entre los laboratorios. Se concluyó que los protocolos para la preparación y determinación de la edad de los otolitos eran en general coherentes. Los resultados preliminares de estudios recientes de marcado-recaptura con oxitetraciclina sugieren que los recuentos del incremento diario podrían ser útiles solo para peces muy jóvenes, mientras que los incrementos anuales parecen depositarse anualmente durante toda la vida. Se encontró un patrón similar anteriormente en un estudio de marcado-recaptura con cloruro de estroncio sobre el patudo. Las estimaciones de edad máxima de 16-18 años para el rabil y el patudo han sido validadas con una datación por bomba de carbono 14. El Grupo acordó un protocolo estandarizado para asignar el tipo de borde del otolito y para que solo las zonas opacas plenamente formadas se cuenten como anillos. Se presentaron algoritmos de determinación de la edad para estimar la edad fraccional. Los participantes concluyeron que los actuales protocolos de estimación de la edad anual son válidos, y que la determinación de la edad de ambas especies usando recuentos de incrementos anuales de crecimiento es posible y recomendada para garantizar que la edad de los ejemplares más mayores no está siendo sistemáticamente subestimada y para reflejar la variación en la longitud por edad.

KEYWORDS

Yellowfin tuna, bigeye tuna, otolith, ageing

1. Rational for workshop

Inter-laboratory ageing comparisons are an important quality control measure to reduce the potential for age bias among studies. One of the first steps is to directly compare and evaluate ageing methods among laboratories to ensure consistency in otolith preparation and reading protocols. The exchange of otolith reference collections among laboratories can follow to formally estimate precision and bias. This work should be undertaken in addition to the validation of the direct ageing method.

Otolith-based annual ageing protocols were recently validated for yellowfin and bigeye tuna caught in the Gulf of Mexico using bomb radiocarbon (14C) dating (Andrews *et al.* 2020). Previously, annual ageing protocols for bigeye were validated in the western Pacific Ocean via a strontium chloride mark-recapture experiments (Farley *et al.* 2003; 2006), with additional validation undertaken on bigeye and yellowfin tuna more recently (Farley *et al.* 2019). Work is also being undertaken to validate annual ageing protocols of bigeye and yellowfin tuna in the Atlantic Ocean via an oxytetracycline mark recapture (Ailloud *et al.* 2019). Given the number of laboratories now ageing bigeye and yellowfin tunas using 'annual ageing' methods, it was important to hold a workshop of ageing experts to improve the inter-comparability between ageing data sets and ensure consistency across international agencies when ageing these two species. The workshop allowed participants to share knowledge, methodologies and assess the different aspects of ageing determinations that contribute to discrepancies in datasets.

After opening remarks and introductions the workshop began with presentations summarizing ageing program.

2. Presentations and Discussions

2.1 Overview of the National Marine Fisheries Service Panama City Laboratory ageing program - Robert Allman

This presentation discussed the history of the ageing program at the NMFS Panama City Laboratory and described current sampling programs for tuna species. The Panama City Ageing program archives ageing structures from over 100 species from the US Gulf of Mexico, Atlantic and Caribbean collected from commercial and recreational landings and fishery-independent surveys. Some of these collections go back as far as the 1970s, but most have been collected since the early 1990s. Historically, ageing has been concentrated on 6 species due to their economic importance in the Gulf of Mexico. These include 2 shallow water serranids, gag and red grouper, 2 lutjanids, red and vermilion snapper and 2 scombrids, king and Spanish mackerel. On average we receive several thousand otoliths per year for each of these 6 core species. Of these species the most effort is spent on red snapper which is the most valuable finfish in the Gulf of Mexico. Annually about 12-15 thousand red snapper otoliths are received and about 5 thousand are aged. Twelve other reef fish associated species make up the majority of the remaining effort spent on ageing. For these 12 species we typically receive several hundred otoliths per species per year.

Tuna ageing has been conducted at the Panama City laboratory since 2010. Samples are obtained from 5 programs. The NOAA Pelagic Observer Program (POP) obtains biological samples including otoliths, gonads and other tissues through at-sea observers aboard commercial long-line vessels. POP concentrates on bluefin tuna, but other pelagic species are sampled opportunistically. Quantitative Technologies for Research and Analysis (QuanTech) is contracted to sample otoliths, gonads and other tissue samples from the U.S. Atlantic with most effort concentrated in the Mid-Atlantic states. Samples are collected through opportunistic dockside sampling of the recreational/tournament fishery targeting bluefin tuna with other tuna species sampled as time permits. The North Carolina Division of Marine Fisheries samples bluefin tuna otoliths during the winter months as bluefin migrate south off North Carolina. The NOAA Northeast Regional Office has provided otoliths from bluefin tuna from the commercial fishery off New England. Most of our yellowfin tuna samples (otoliths and gonads) are provided by a port sampler stationed in Venice, LA who samples the recreational fishery via opportunistic dockside sampling. This region sees the largest catches of yellowfin tuna in the Gulf of Mexico. Exact catch locations were usually only available for fish collected from the commercial fishery.

Tuna species are sampled in smaller numbers compared to most other species. Bluefin tuna have been sampled since 2009 and average about 300 otoliths sampled per year. Yellowfin tuna sampling has been more haphazard with approximately 1600 otoliths collected since 2004. Bigeye tuna are sampled infrequently with only 15 samples recorded since 2011.

2.2 Overview of Commonwealth Scientific and Industrial Research Organisation ageing program - Jessica Farley

This presentation provided a brief overview of the pelagic fisheries research undertaken by CSIRO Oceans and Atmosphere, including studies on population biology. One focus of the research has been the development of methods to estimate the age of tunas and billfish using otoliths, which has included studies on southern bluefin tuna, albacore tuna, bigeye tuna, yellowfin tuna, broadbill swordfish and striped marlin. Research on southern bluefin tuna has been a focus for several decades and it was the first tuna species where the accuracy of annual ageing methods was confirmed using bomb radiocarbon techniques.

A summary was provided on research to estimate the age and growth of bigeye and yellowfin tunas in the Western and Central Pacific Ocean (WCPO). For bigeye tuna, a preliminary composite growth model was developed by the Pacific Community (SPC) in the early 1990s based on counts of assumed daily growth zones in otoliths and analysis of tag-return data. Validating the accuracy of the daily age estimates was required and a series of strontium chloride mark-recapture experiments were subsequently conducted in the Coral Sea (southwest Pacific) by SPC and CSIRO. Analysis of marked otoliths from recaptured fish indicated that daily age estimates are not reliable for fish 72 to 109 cm fork length, but it showed that annual age estimates were reliable for fish aged 2 to 9 years (Farley *et al.* 2003; 2006). Given this validation, a large study to estimate the annual age of bigeye in the Australian region was undertaken in the 2000s (Farley et al 2006).

Over the last decade, the Western and Central Pacific Fishery Commission (WCPFC), CSIRO and SPC has funded a large regional study of bigeye tuna age and growth in the WCPO with over 1200 otoliths analysed. The majority of otoliths were obtained from the WCPFC Tissue Bank. A study that compared daily and annual age estimates using 'sister' otoliths from the same fish was also completed; this showed that annual ages were generally higher than daily age for fish older than two years (Williams *et al.* 2013). All otoliths are read by FAS using the methods developed by CSIRO in the 2000s. Multiple transverse sections are prepared from each otolith and two counts of the opaque zones are undertaken before a final count is assigned. Opaque zones are only counted if completely formed and translucent material is visible after the zone. An edge type is classified to each otolith reading as narrow (new opaque), intermediate (narrow translucent) or wide (wide translucent). A readability score is also assigned (0-5). An image of each otolith section is taken, and the opaque zones are marked and measured. Daily ageing was used to confirm the location of the first opaque zone. An algorithm is used to convert counts of opaque zones to a fractional (decimal) age using a nominal birth date, the capture date and the state of completion of the marginal increment of the otolith. Maximum age estimates of 12-14 years have been obtained.

Recently, the WCPFC and CSIRO funded a study to estimate the age and growth of yellowfin tuna in the WCPO. Preliminary analysis of 40 otoliths and fin spines (30 - 172 cm FL) indicated that otoliths are a suitable structure for estimating annual age and (preliminary) maximum ages of ~13 have been obtained. As expected, fin spines showed resorption and vascularization leading to a “loss” of early increments and were not suitable for annual age estimation beyond three years. As found in bigeye, a comparison of daily and annual age estimates using ‘sister’ otoliths from the same fish showed that annual age was generally higher than daily age for yellowfin tuna. Analysis of a small number of strontium chloride mark-recapture otoliths indicate that counts of daily growth zones may not be a reliable source of age information for yellowfin > age 1, while counts of annual growth increments appear to be reliable. A total of ~1600 otoliths have been selected for analysis, which should be completed in late 2020.

2.3 Overview of Fish Ageing Services (FAS) - Kyne Krusic-Golub

This presentation provided an overview of Fish Ageing Services (FAS) and outlined the methods used for routine age estimation of tunas. Fish Ageing Services provides a fee for service facility in the field of fish and shark age estimation. The services provided include: preparation of hard parts (otoliths, spines and vertebrae), method development, routine age estimation, analysis of otoliths from marked and recaptured samples, daily age estimation and image analysis (measuring increment widths for chronology, growth relationships to environmental drivers, marginal increment studies). Fish Ageing Services have worked with a wide range of clients, both nationally and internationally, and current staff within FAS have a combined experience in the age estimation field excess of 50 years. Work has been completed on more than 250 different species, including 8 species of tuna and 3 species of billfish. The number of age estimates produced by FAS each year ranges between 14,000 to 20,000 and the current sample archive is close to 440,000 in total.

Most samples processed at FAS are for routine age reading with many species requiring several hundred samples to be prepared and read each year. Therefore, methods used at FAS were developed to allow efficient and cost effect preparation and reading of large volumes of samples. To facilitate the cost and time savings, methods were developed to allow for multiple transverse sections of multiple otoliths to be cut at the one time.

A summary of this method was provided to the group (see section 3.1 below). For southern bluefin, bigeye, albacore and yellowfin tunas the multiple sectioning method is used. The only variation is that for yellowfin tuna, the section thickness is increased to 350 µm. Due to their small size and brittle nature, billfish otoliths are prepared individually using hand grinding techniques to provide thin transverse sections approximately 200 µm thick. For Atlantic bluefin tuna otolith preparation follows the established protocol where the otoliths are prepared individually, and two sections are cut through the otolith centers using an Isomet cutting saw (Buehler 1000©).

While FAS was the only group within this workshop that cut serial sections from multiple otoliths, the group all agreed that transverse sectioning of sagittal otoliths was the preferential method for preparing yellowfin and bigeye otoliths for annual age reading. Advantage and disadvantages in preparing multiple sections were presented and discussed during this presentation and are listed below.

Advantages

- More time/cost efficient.
- Multiple sections increase the chance that at least one section within a sample will contain the primordium.
- Multiple sections for reference when ageing allowing the age reader can use multiple sections to come up with an age estimate.
- Sometimes one of the sections adjacent to the primordium section can be easier to read than the primordium section. Clearer than the primordium section, or perhaps it is just cleaner in a certain area within the section.
- Diamond blades used in this process are relatively cheap (US$35.00 per blade).

Disadvantages

- Requires a highspeed saw with custom setup.
- Not preferred for microchemistry or daily ageing.
- Cuts in a straight line so if the otoliths aren’t embedded straight, then some otoliths will not be cut close to the primordium.
- If there is an issue with the machine or user error when cutting, then potentially 5 samples could be affected rather than just the one if using the single section method.

List of equipment used in the multiple section method

- Moulds - Custom Silicon Rubber (Elastosil M4670a & b).
- https://dalchem.com.au/wacker-m4670.html
- Polyester resin & Methyl Ethyl Ketone Peroxide (MEKP).
- https://www.barnes.com.au/polyester-resin/clear-casting-resin-1267
- https://www.barnes.com.au/polyester-resin/mekp-k1285?search_query=MEKP&results=9-
- Highspeed saw (modified GEMMASTA trim saw).
- GEMMASTA are no longer available (Covington, Rock Rascal and Lortone do produce trim saws that could be modified).
- 4 inch Pro Slicer diamond blades – 4 x 00.4 x 5/8.
- https://www.johnsonbrotherslapidary.com/P4-4-58.html

2.4 Overview of Louisiana Department of Wildlife and Fisheries ageing program - Erik Lang

LDWF began a yellowfin tuna research initiative in 2012, which was composed of both funded extramural projects and research projects led by LDWF biologists. Funded extramural projects were based on biological sampling conducted by LDWF biologists at recreational fishing marinas in Louisiana.

From 2012 through present LDWF has sampled the Charter fishery that operates in Venice, Louisiana. Curved fork length is measured to the nearest millimeter and whole body weight to the nearest 0.1kg. If whole body weight is not available, then weight is not recorded. Sagittal otoliths are removed and cleaned carefully with water and forceps. The cleaned otoliths are stored in small plastic centrifuge tubes and inserted into labeled envelopes. Sex is identified macroscopically.

Initially, no ageing work was funded or undertaken. However, when the importance of updated ageing data became clear, and the data gap for yellowfin tuna ages from the Gulf of Mexico was identified, LDWF biologists collaborated with Texas A&M University Galveston (TAMUG) Fisheries Ecology Lab to develop updated methods for using annuli to age yellowfin tuna from the Gulf of Mexico. Beginning with a workshop at TAMUG in February of 2015, LDWF and TAMUG developed a yardstick based on daily increments to identify the first annulus, following methods previously applied to annual ageing of Atlantic bluefin tuna by Secor *et al.* (2014). As discussed in Lang *et al.* (2017), the yardstick was applied to collections of yellowfin tuna from the Louisiana recreational fishery, generating a novel growth curve and questioning contemporary estimates of growth and longevity in yellowfin tuna. Since then, LDWF biologists have collaborated with NOAA-Panama City to combine yellowfin tuna ageing datasets to produce a consensus reading protocol, yellowfin tuna ageing reference set, and combined growth curve, as well as validation of the annual ageing technique as presented in Andrews *et al.* (2020). Collection and ageing of yellowfin tuna otoliths using the recently developed technique continue, with LDWF collecting ~350 otolith sets annually as part of a current project to document age structure in the Louisiana recreational yellowfin tuna fishery. In total we have collected 2,635 sagittal otoliths pairs from 2012-2019.

2.5 Overview of University of Maine Tuna Research – Riley Austin

Current research in the Pelagic Fisheries lab focuses on Atlantic bluefin, yellowfin, bigeye, and albacore tunas, white and blue marlin, swordfish and round-scale spearfish. The lab's focus is on furthering our understanding of life history to improve biological inputs in stock assessments and reduce uncertainty to produce impactful management advice. Specific research projects include age validation, age and stock structure, spawning dynamics, movement/migration, energetics, post-release mortality, and foraging ecology.

Biological Sampling - Beginning in 2010, the lab has had a biological sampling program which supplies not only its research programs, but the programs of many other institutions interested in doing research on highly migratory species. Samples are collected from rod and reel, purse seine, pelagic longline, and harpoon fisheries. If possible, muscle tissue, sagittal otoliths, liver, eyes, stomachs, dorsal spines and gonads are collected from each fish. Some samples are used for existing projects with remaining or unused tissues archived in the lab for future use. To date, approximately 9,000 bluefin, 1,000 bigeye and yellowfin tunas, 80 blue and white marlins (including round-scale spearfish), and 60 albacore tuna are in the lab archive.

Age and Growth - Current projects on age and growth include bluefin, bigeye, yellowfin, swordfish, blue and white marlin (inclusive of round-scale spearfish). The projects for these species are in different phases. Annual formation of increments in Atlantic bluefin sagittal otoliths has been validated and otolith collections have occurred for some time. The lab participates with other laboratories across the Atlantic to provide NMFS/ICCAT with production aging for commercial and recreationally landed Atlantic bluefin tuna from all existing gear types. Yellowfin and bigeye tuna ageing in our lab is more recent, within the past couple of years. In collaboration with NMFS, and the LDWF, the University of Maine supported an age validation study for yellowfin and bigeye to confirm that each annulus does in fact represent one year of life. This confirmation allows for the use of sagittal otoliths and their annuli to be counted as an estimate of age. This workshop (one of hopefully many) is to begin standardizing the use of sagittal otoliths for age estimates. We have begun preliminary work on ageing blue and white marlin (and round-scale spearfish) by comparing age estimates from sagittal otoliths and spines. Like marlin, bluefin spines are used to estimate age of fish sampled in the eastern Atlantic Ocean, but spines from both species are prone to the loss of internal rings due to the vascularization of the spine core. These losses can be corrected using back-calculation, via measurements of younger specimens' rings prior to resorption, but in bluefin the spines appear to underestimate ages beyond the 12th year of life. Thus, we are investigating if this same underestimation occurs in marlins sampled off the northeastern U.S. and if so the level of bias associated with those counts.

3. Otolith Processing Methods

3.1 Fish Ageing Services – Kyne Krusic-Golub

Clean and dry otoliths are embedded in rows of five in blocks of Polyplex ortho clear casting resin, ensuring that the primordium of each otolith is in line so that each line of otoliths can be cut on the transverse axis. The resin blocks are left to cure for at least 2 weeks prior to cutting. A minimum of four serial sections are cut from the centers of the otoliths using a modified Gemasta™ lapidary diamond cutting saw fitted with a 250 µm wide diamond impregnated blade. The section thickness is dependent on the species however, we have found that for most of the species that we work with, sections approximately 300 µm thick are preferred. Sections from each sample are then cleaned, dried and mounted on clear glass microscope slides (50 x 76 mm) under glass coverslips using resin. Using this method up to five otoliths can be prepared for ageing at one time and using this method approximately 250 otoliths per day can be cut and mounted on slides. The method has been used in routine ageing of other tuna species and is shown in detail in the manual for the age determination of southern bluefin tuna, *Thunnus maccoyii* (Anon, 2002).

3.2 University of Maine - Riley Austin

Otolith processing and age estimation was based on methods developed by Busawon *et al.* (2015), modified by Rodrigues-Marin (2019). After extraction otoliths are dried and stored in vials. Should any remaining matrix material be found after storage it is cleaned with an ultrasonic jewelry cleaner. Whole sagittal otoliths (left and right) are weighed and imaged, then embedded using Epothin 2 Epoxy hardener and resin at a 17:40 ratio respectively. Once the resin hardens transverse sections are cut using an Isomet 1000 low-speed saw with diamond edged Buehler blades. A single 1.0 mm section cut on either side of the origin provides a rostral 'V' and an antirostral 'Y' section. Sections are mounted to glass slides using QuickStick Mounting Wax. After mounting, sections are polished using 600, 320, and 180 grit sandpaper. A final polish is used with a felt pad containing a light coating of water mixed with MicroPolish 2 Alumina powder. Otolith sections are imaged under a compound microscope and scale bars are created on each image to provide the reader guidance on the approximate location of first annulus formation. These scale bars were created through a workshop held at the University of Maryland (Dr. Dave Secor) and created by Secor's measurements of ventral arm length from one-year old bluefin tuna. Following digitizing, all aging is conducted using Adobe Photoshop annotating each annulus on the image with color coded spots.

3.3 National Marine Fishery Service Panama City Laboratory - Ashley Pacicco

All whole otoliths are weighed and imaged using Infinity Analyze software. Otolith preparation followed established methodology for Atlantic bluefin tuna (Secor *et al.* 2014). First, otoliths are embedded using a mixture of 30 ml Epofix™ resin to 4 ml of hardener. The otolith is placed distal side up in the center of each mold and set to dry for 12 to 24 hours. The otolith core is marked using a dissecting scope between 10 to 15× under reflected light (**Figure 1**). The embedded otolith is placed in the arm of an Isomet low-speed saw, with the sectioning plane positioned between two 4-inch diamond encrusted blades. The resulting single section is ~ 0.5 mm thick. Otolith sections are mounted post-rostrum side up using Crystalbond™ mounting medium on a glass slide. Cytoseal™ is applied over the section to fill in any scratch marks from sectioning.

3.4 Louisiana Department of Wildlife and Fisheries - Erik Lang

The left otolith of each fish is marked on the end of the anti-rostrum with a permanent marker. The otolith is embedded in a 5:1 mixture of Huntsman Araldite® and Aradur® and sectioned transversely. The marked tip of the anti-rostrum is used to orient the otolith on the center blade of a three-blade setup on a Buehler low speed Isomet saw. This results with a y-section and a v/y-section that has a small amount of the anti-rostrum. Sections are cut to 0.535mm thickness, mounted on a slide with Locktite® UV glue and covered with Shandon® mounting media.

4. Near Infrared Spectography

Beverly Barnett (National Oceanic and Atmospheric Administration/National Marine Fishery Service/ Southeast Fisheries Science Center Panama City laboratory) presented results of her research using Near Infrared Spectroscopy: Estimating Age with Fourier Transform Near Infrared Spectroscopy.

NMFS laboratories from five regions across the United States are currently investigating the utility of Fourier transform near infrared spectroscopy (FT-NIRS) as a rapid and more efficient method for estimating fish age. The impetus for this investigation stems from the fact that while there is an increasing demand by Fishery Management Councils for more stock assessments, the ability to meet these demands is restrained when relying on conventional, labor-intensive, and time-consuming methods for providing age estimates. Therefore, there is a need for a rapid method to estimate fish age given the laborious and time-constraining process currently used in aging laboratories. FT-NIRS is a well-established quality control method utilized by food and agriculture, chemical, petrochemical and pharmaceutical industries. More recently, it has been applied as a novel method for aging fish otoliths and shark vertebra. Fish otoliths, which continue to grow throughout the life of the fish, are composed of calcium carbonate ($CaCO_3$) deposited on a protein matrix. FT-NIRS quantitatively measures the absorption of near infrared energy (wavelengths 800 to 2,500 nm) in material that contains covalent bonds such as the protein molecules in otoliths. Preliminary results suggest that the spectral information most important for otoliths are the regions related to the molecular constituents (or functional groups) in proteins, such as carbon-hydrogen (C-H), oxygen-hydrogen (O-H) and nitrogen-hydrogen (N-H) groups. A critical component for successful implementation of FT-NIRS relies on building calibration and validation age prediction models, where age estimates assigned by either an individual reader or consensus age (i.e., age agreed on by multiple readers) provide the input age data for the predictive model. In the northern Gulf of Mexico, this technology has recently been applied to otoliths from red snapper, *Lutjanus campechanus,* and preliminary results show good agreement between the FT-NIR predicted age and traditional observed age. Additional research and development are required before this technology can be implemented.

5. Age Validation

The group discussed options for validating ages including mark-recapture experiments using oxytetracycline (OTC) and strontium chloride ($SrCl_2$). OTC use is often problematic due to health and safety regulations. $SrCl_2$ was suggested as another option, however, a scanning electron microscope (SEM) is required to detect the validation mark.

Results of validation studies were presented.

5.1 The Atlantic Ocean Tropical Tuna Tagging Programme (AOTTP) – oxytetracycline marking and otolith reference collection - Lisa Ailloud

The ICCAT Atlantic Ocean Tropical Tuna Tagging Programme (AOTTP) is a 5-year project funded by the European Union (90%) and other ICCAT Contracting and Cooperating non-Contracting Parties (CPCs; 10%). The ultimate objective of the programme is to contribute to the food security and economic growth of the Atlantic coastal states by ensuring sustainable management of their tropical tuna resources. AOTTP activities include collecting tag-recapture and associated data for tropical tuna species across the Atlantic, and training scientists from developing CPCs in tagging, data collection and scientific analyses to ultimately improve estimates of key parameters (e.g. growth, mortality and migration) for stock assessment purposes.

AOTTP has been collecting data on the three main tropical tuna species in the Atlantic Ocean – yellowfin tuna (YFT), bigeye tuna (BET) and skipjack tuna – as well as for two neritic species, little tunny and wahoo. As of December 2019, around 115,000 fish (objective=120,000 fish by 2020) have been tagged with conventional tags in the exclusive economic zone (EEZ) of 21 countries and also the high seas, and 16,000 tagged fish have been recovered by tag recovery teams across the Atlantic. In addition, over 500 electronic tags (satellite and archival tags) have been deployed. So far, data have been recovered for 108 of these tags. Tag recovery offices are, for the most part, autonomous teams that answer directly to ICCAT AOTTP and oversee raising awareness, collecting data on recovered tags, monitoring docks for tags, entering data in the AOTTP ICCAT database and paying rewards. Since 2016, 20% of all tagged fish have been injected with OTC and marked with a red tag. When a red tag is recovered, tag recovery officers are instructed to purchase the fish from the fishermen and dissect it for basic biological data (e.g. species, sex, maturity, etc.) and extract hard parts (otoliths, spines and vertebrae). So far, 1068 BET and YFT have been recaptured and, of those, 835 were purchased. To date, 365 YFT and 265 BET have been dissected and otoliths successfully extracted.

AOTTP has multiple ongoing age and growth projects. 1. An otolith reference collection based on readings of daily increment has been created by teams in Senegal and Brazil and is currently being finalized. 2. Fish Ageing Services (FAS) was contracted to analyse ninety paired otoliths of OTC marked fish, including annual and daily ageing as well as validation work. A preliminary analysis of 16 OTC marked YFT otoliths (annual increment counts) and four sister otoliths (daily micro-increment counts) was presented at the last ICCAT YFT data preparatory meeting. Increment counts were compared to known times at liberty to validate the deposition rate of ("daily") micro-increments and larger ("annual") increments. Preliminary results suggest that age estimates based on daily increment counts may lead to underestimation of age, while annual increments appear to be deposited on an annual basis. More complete results will be available soon. 3. A suite of training workshops was conducted to mentor two age reading technicians in Dakar and Abidjan who are now in charge of analysing AOTTP samples. 4. A scientific consortium is currently working on modelling growth for the three main tropical tuna species in the Atlantic from AOTTP tagging data and hard parts. They welcome any institution interested in collaborating and contributing their hard part data to the effort.

With respect to growth modelling, there are two issues that require further investigation. The first concerns variability in size at age and the second is in regards with the compatibility of daily and annual readings. During the last ICCAT YFT assessment, it was noted that the YFT otolith data from USA/Gulf of Mexico showed very high levels of variability in size at age. AOTTP suggests exploring whether the age adjustment algorithm is contributing to this perceived variability and/or whether otolith weight information could be used to improve the assignment of a decimal age to the counts. Regarding the differing reading methods (daily vs. annual), it will be important to determine exactly where the divergence in age estimates occurs for each species so we can selectively exclude certain daily samples from the analysis. Only daily ages from very young fish, where daily counts are deemed reliable (i.e. before the divergence) should be included, and this should be done in such a way as to minimize potential biases.

5.2 Validated longevity of yellowfin and bigeye tuna of the northwestern Atlantic Ocean - Robert Allman

The age, growth and longevity of yellowfin and bigeye tuna remain problematic in that attempts to validate age estimates have been typically incapable of evaluating maximum age. Otolith growth zone structure can be complicated for tropical pelagic fishes because they live in a more a seasonal environment than higher latitude habitats. However, bomb radiocarbon (^{14}C) dating has evolved considerably over the last 25 years and is a well-founded approach that has been useful in accurately describing the life history characteristics of several tropical pelagic species. In this study, age was estimated by counting opaque zones in thin sectioned otoliths with transmitted light. Typically, the first 5-7 opaque zones are broad and diffuse. The distance to the first opaque zone was verified using daily increment counts. Ages beyond 7 years were compressed and were often difficult to distinguish. Maximum age estimates of 16–18 years for YFT and BET of the northwestern Atlantic Ocean were validated with bomb ^{14}C dating. A novel aspect of the method is use of the ^{14}C decline period (more recent than ~1980) — after nuclear testing and as described by regional coral records of the Gulf of Mexico — to provide valid estimates of age through ontogeny. Yellowfin tuna aged 2 to 18 years (n = 34, 1029 to 1810 mm FL) led to birth years that were coincident with the bomb ^{14}C decline reference. While BET aged 3 to 17 years were fewer (n = 12, 1280 to 1750 mm FL), the series was also in agreement. Results indicate there was no age reading bias and that age discrepancies for previous studies may have led to truncated estimates of longevity.

5.3 Validation for bigeye and yellowfin tuna in the Western and Central Pacific Ocean - Jessica Farley

In the western Pacific, CSIRO and the Pacific Community (SPC) began work to validate daily and annual zone formation in otoliths of bigeye and yellowfin tuna in the early 1990s through analysis of strontium chloride mark-recapture otoliths. Tagging programs were undertaken in the Coral Sea where bigeye and yellowfin tuna were captured, injected with a strontium chloride solution and released. By the early 2000s, otoliths from 10 bigeye tuna had been analyzed from fish that had been at liberty between 0.7 and 6.6 years. The strontium marks in the otoliths were located using a scanning electron microscope (SEM) and the number of annual zones visible after the mark was counted using a light microscope. In all cases, the number of annual zones after the mark was equal to or within the range expected, given the time at liberty. For seven fish, the number of daily growth zones visible after the mark was counted in sister otoliths using both the SEM and light microscope. All daily zone counts underestimated the days at liberty; the difference was 7.7% to 30.0% lower (72-129 cm FL). The study concluded that the annual periodicity of increments for bigeye age 2 to 9 years was validated, but the daily periodicity of growth zones for bigeye > age ~1 was not validated.

In 2019, additional daily age validation work conducted by CSIRO and Inter-American Tropical Tuna Commission (IATTC) prior to and during a workshop on yellowfin and bigeye age and growth held at IATTC labs in La Jolla in August 2019. Paired otoliths (left and right) were analysed from four fish (two bigeye and two yellowfin) marked with $SrCl_2$ in the Coral Sea and recaptured 61 to 427 days later. Frontal sections were prepared from each otolith. One otolith of each pair was prepared and the strontium mark was located using the SEM. The 'sister' otolith was prepared by IATTC and the expected position of the strontium mark was located using high-resolution images of the otolith section from the SEM. Counts of daily growth zones were made from the mark to the otolith tip and compared to days at liberty. In all cases, the number of daily zones counted underestimated the days at liberty; the difference was 24.5 to 56.1% lower. The results indicate that daily growth increments are not a reliable source of age information for yellowfin >74 cm and bigeye >82 cm in the WPO. An OTC marked yellowfin tuna (145 cm FL) otolith from the Atlantic Ocean (collected through the ICCAT AOTTP) was also analysed by IATTC. The number of daily zones counted underestimated the days at liberty by 60.8%.

Preliminary annual age validation for WPO yellowfin tuna was also undertaken using $SrCl_2$ marked otoliths. The number of annual zones observed after the mark was counted and compared to the days at liberty. One fish was only at liberty for 61 days and subsequent growth was not enough to validate annual ageing. The other two yellowfin were at liberty for 261 and 375 days, and one opaque zone and one translucent zone was visible after the mark. This result suggests that counts of annual growth zones in otoliths may be a reliable source of age information for yellowfin in the WPO.

6. Edge type discussion

The group agreed to follow the protocol used by Farley *et al.* (2016) for assigning an edge type moving forward: opaque edge, narrow translucent zone (≤1/3 of previous translucent zone) and wide translucent zone (>1/3 of previous translucent zone) (**Figure 2**).

7. Ageing algorithm

Methods for assigning calendar and biological age were presented.

7.1 Ageing protocols for calculating calendar and biological age used for Gulf of Mexico yellowfin tuna - Ashley Pacicco

Standard ageing protocol followed the established criteria used for Atlantic bluefin tuna (Busawon *et al.* 2015). An annulus was identified as a successive opaque and translucent zone under transmitted light. The ventral arm was used for ageing and the dorsal only used to verify ventral counts. The first 1-5 opaque zones were identified as wide, becoming narrower as the age estimate increased. A ~ 1 mm measurement was made from the core along the interior margin of the ventral arm and was used as a guide for determining the location of the first annulus (Lang *et al.* 2017). Each otolith section was aged twice by a single reader with at least 2 weeks between readings. If the 1st and 2nd reads differed, a 3rd read was conducted to determine the final ring count. A recently developed ageing protocol for Atlantic bluefin tuna (Rodríguez-Marín *et al.* 2020), which only counts fully formed opaque zones (i.e. must have translucent margin for last opaque zone to be considered fully formed) has been adopted for yellowfin tuna by the NMFS ageing group.

To determine calendar age, 1 year was added to the opaque ring count if a large translucent margin (>1/3 to full complete) was assigned with a catch date between 1 January and 31 July. For all other capture dates and edge types, opaque ring count equals age. Fractional year was determined using the equation:

$$\text{Fractional year} = [(\text{capture date} - \text{date of peak spawning})/365]|$$

Where the date of peak spawning =1 July. To determine a final age, the fractional year was added to the calendar age if the capture date was after 1 July or subtracted if before (Allman and Goetz 2009). An assigned birth date of 1 July was selected for yellowfin tuna because it corresponded to the midpoint of the peak spawning season in the Gulf of Mexico (May to September) using a gonadosomatic index (GSI) for males and females (Brown-Peterson *et al.* 2013).

7.2 Louisiana Department of Wildlife and Fisheries ageing methods - Erik Lang

We take a picture of each otolith under an Olympus SZX12 dissection scope with a five megpixel LAXCO® SeBaCam™ camera. An estimated "yardstick" that measures the distance to the first increment is placed on each picture. The otolith is aged through the picture on the computer screen, but fuzzy or difficult increments are taken to the microscope for better observation. Measurements are taken from the bottom of the core area to the bottom of each annual increment, and to the otolith edge, to the nearest 0.0001 millimeter. Edge codes are determined through the marginal increment ratio (MIR) calculated from these measurements. For fish age 3 and older the distance between the last opaque zone and edge is divided by the previous distance between increments. For ages less than 3, the distance to the otolith edge from the previous increment is divided by the third quartile of the distances between the first and second increment for age 1 and the second and third increment for age 2. For instance, the third quartile of distances between the first and second increment in 2018 was 0.52mm. Therefore, if an age 1 marginal distance was 0.25 to the edge then:

MIR=0.25/0.52=0.48

Edge codes were then assigned according to MIR.

Edge code 1 is <0.333

Edge code 3 is 0.333-0.666

Edge code 4 is >0.666

Calendar and Fractional Age - Based on a joint decision with the NOAA Fisheries Lab in Panama City, Florida, to determine calendar age, 1 year was added to the age of fish if the otolith edge codes was 3 and 4 and the fish was caught between January 1st and July 31st. For example, an age 4 fish with an edge code of 4 and caught in April was revised to a 5, but if that same fish was caught in October, it would remain as an age 4. To estimate fractional age, an assumed birth date of July 1st was used. A year was subtracted from ages with edge code 1 and a year was added to ages with edge codes 3 or 4 for fish caught after the assumed birthdate but before October 1. The fraction of a year from the birthdate prior to the capture date was then added to the adjusted age. For example, if an age 4 yellowfin tuna with edge code 1 was caught on April 1st, the fractional age would be 3.75 years. Conversely, if an age 4 with edge code 4 was caught on August 1st, the fractional age would be 5.09 years (32 days past the July 1 birthdate, so 32/365ths or 0.09 of a year).

7.3 The ageing algorithm for calculating a biological age for bigeye in the Western and Central Pacific Ocean (WCPO) - Jessica Farley

An ageing algorithm was developed.

for bigeye tuna in the WCPO to estimate a decimal (fractional) age from the counts of annual opaque zones in sectioned otoliths. The algorithm uses a nominal birth date, the capture date and the state of completion of the marginal increment (edge classification) of the otolith. The algorithm

$$a = (n + b) + \frac{r}{365}$$

where *a* is the decimal age, *n* the count of opaque zones (only counted if completed), *b* the count adjustment based on otolith edge type and month of capture (**Table 1**), and *r* the catch date (expressed as number of days since the nominal birth date).

The nominal birth date used is 1 July, although other options were considered including a random birth date. Based on marginal increment and edge type analysis, it was determined that opaque zones are completed in the period between April and September, and 1 July is used as the point for adjusting the counts of opaque zones to assign individuals into their correct age class. In short, for fish caught between April and September, the count of opaque zones is adjusted depending on whether the zone has been deposited and counted, or not. The otolith edge type is used to decide based on the **Table 1**. This part of the algorithm places the fish into the correct age class. The number of days between the last birthday and capture day is added to the age class to provide a decimal age. A working example of the algorithm was presented, which showed that the same decimal age is obtained for fish born and caught on the same dates even if they have different zone counts and otolith edge types due to the individual variability in the information.

8. Yellowfin and bigeye tuna group ageing exercise

YFT and BET otolith images were projected and aged by group consensus.

The group raised concerns about the large variability in length at age for yellowfin tuna, especially between ages 1-4. Otolith sections from both large and small fish within an age class were aged as a group. No ages were changed from the initial age assignment so it was determined that the variability in length at age could be real and that no obvious ageing biases were occurring between Louisiana Department of Wildlife and Fisheries, Panama City or Fish Ageing Services. The variability in length at age was also noticeable in the varying sizes of otoliths from fish the same length. **Figure 3** and **Figure 4** shows examples of otoliths for Bigeye and Yellowfin Tuna respectively, where large differences in otolith size and age was observed from fish of the same length.

BET sections from NMFS and YFT sections from CSIRO were also aged as a group.

Overall, there was strong agreement on YFT and BET ageing among the group.

9. Recommendations and conclusions from YFT/BET ageing workshop

- Exchange of otoliths among annual ageing experts is needed to ensure consistency in datasets used in the assessment models. For example, an exchange of Ascension Island yellowfin otoliths to be re-read by yellowfin tuna western Atlantic annual agers.
- Caution is warranted when using daily ageing methods past age-1 based upon results from validation work and paired readings in the Atlantic and western central Pacific Oceans. More information will be available once OTC validation work is completed in the Atlantic (AOTTP).
- There is a need for global YFT and BET training and reference sets of annual ages to be made available and exchanged between laboratories.
- Periodic 'annual ageing' workshops should be conducted for YFT and BET to ensure reader precision and standardization.
- Due to the high variability of length at age for YFT and BET, whole otolith weights should be recorded for potential use in growth modeling.
- To ensure consistency in sectioning through the primordium, serial sections towards the post-rostrum of the same otolith is recommended.
- Explore using an age algorithms to adjust for otolith edge type during the opaque zone formation period, as done for BET in the WCPO. Calculate a fractional age based on an assumed birthdate.
- Continue validation of ageing methods including OTC mark-recapture experiments and ^{14}C (bomb radiocarbon) validation techniques.
- Collection of muscle tissue with each otolith for potential genetic stock identification.
- Image all whole and sectioned otoliths, and mark increments counted using the methods of Rodríguez-Marín *et al.* (2020)
- Obtain otoliths from small BET and YFT (e.g. below the minimum size limit in the Western Atlantic (686 mm; 27 inches)) to aid in anchoring the growth curve prior to age-1 using daily ageing methods.

The yellowfin and bigeye tuna ageing workshop provided valuable insights into method development and age estimation protocols between different institutes. Considering that the institutions involved had developed each of their methods in relative isolation, it was encouraging to find that many of the methods for the otolith preparation were similar and that the developed age protocols were in general agreement. The workshop provided the opportunity to work together and discuss first-hand the varying approaches in the preparation of samples, age estimation protocols and the treatment of the resulting age data. While this workshop did not unequivocally provide conclusions that the current annual age estimation protocols are valid, consensus was reached that ageing both species using counts of annual growth increments is possible, and recommended, to ensure that the oldest individuals are not being systematically underaged and to capture the variation in age at length for each age class. Daily ageing can still be useful for small specimens, to help describe growth in young-of-the-year fish and to develop reference scales that aid in detecting the first annual growth increment. The workshop was extremely beneficial and provided great learning and networking opportunity for all who attended.

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Table 1. Opaque zone count adjustment based on capture month (columns) and otolith edge type (rows).

EDGE TYPE	JANUARY TO MARCH	APRIL TO JUNE	JULY TO SEPTEMBER	OCTOBER TO DECEMBER
Wide or Intermediate	0	0	+1	0
Narrow	0	-1	0	0

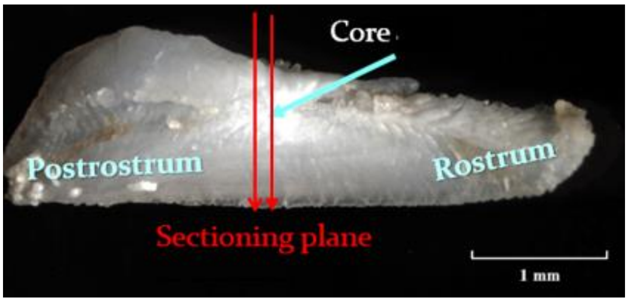

Figure 1. A whole sagittal otolith (left) from a yellowfin tuna at 10× magnification under reflected light. The sectioning plane is denoted by the red arrows.

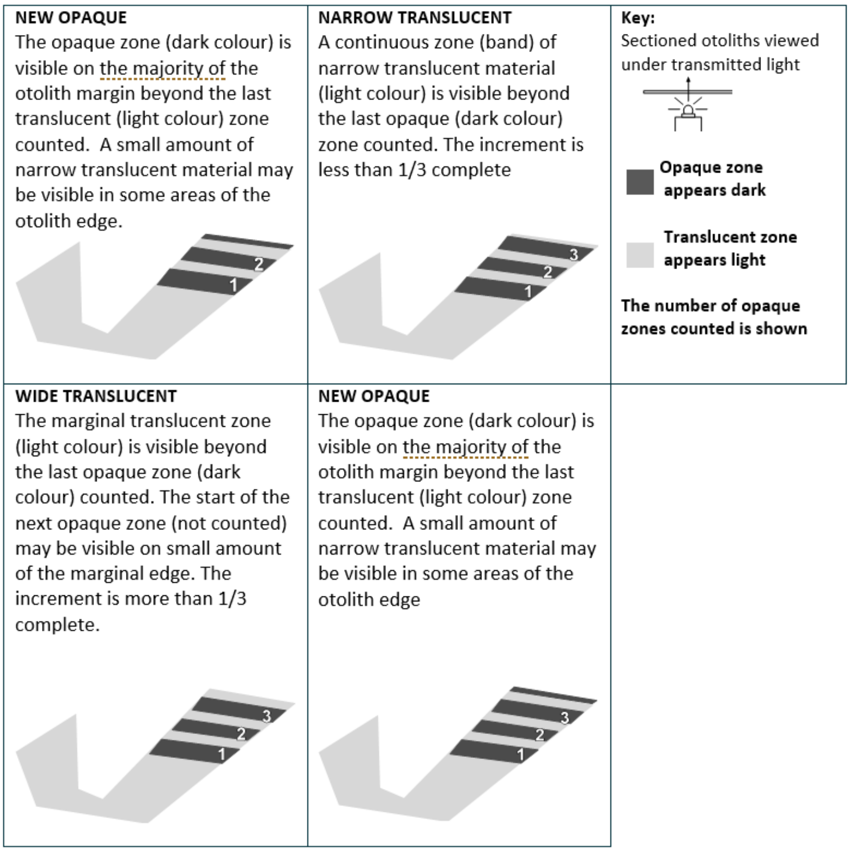

Figure 2. Cycle of edge classification agreed in the workshop. Image from Farley *et al* (2016).

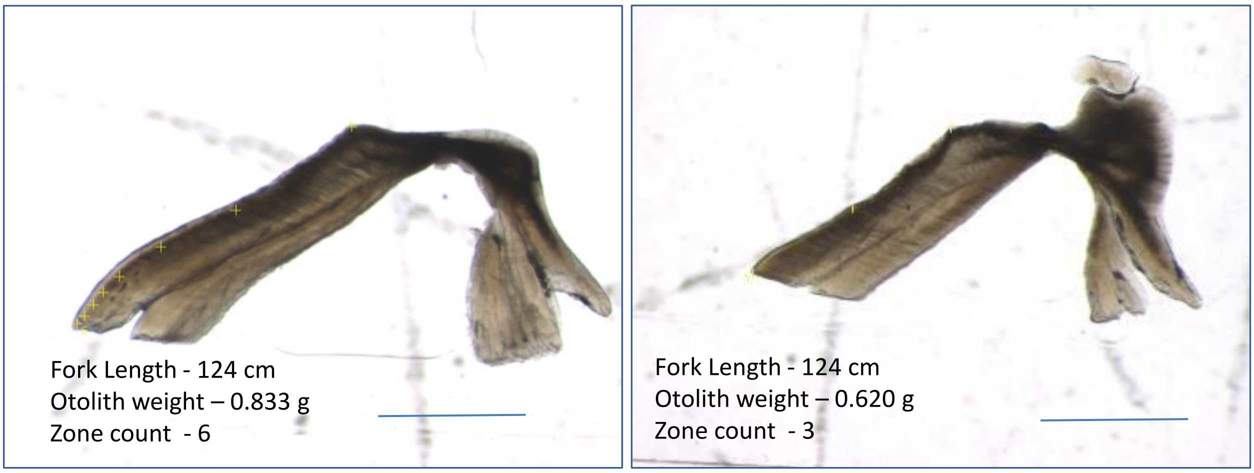

Figure 3. Two examples of Bigeye Tuna otoliths collected from fish of the same length. Scale bar is 1 mm.

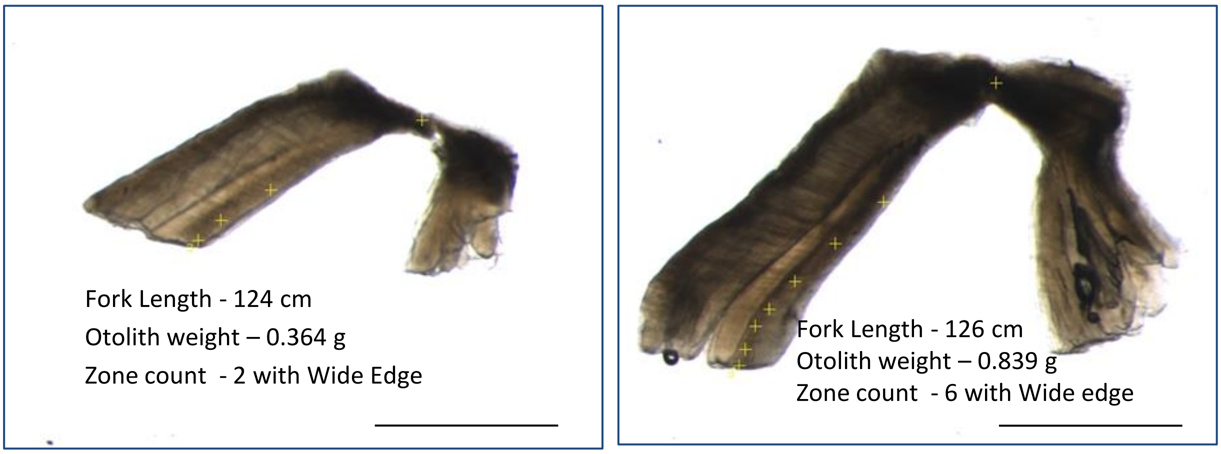

Figure 4. Two examples of Yellowfin tuna otoliths collected from fish with a 2cm difference in length. Scale bar is 1 mm.