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Report on the bomb radiocarbon age validation workshop for tuna and billfish in the WCPO

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Revision 1

- A research plan "Bomb radiocarbon age validation for bigeye and yellowfin tunas in the WCPO" was added to the report (Appendix C).
- Additional text was added to the Summary and Recommendation sections referring to Appendix C.

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Summary

A workshop on bomb radiocarbon age validation for tuna and billfish in the Western and Central Pacific Ocean (WCPO) was held in early July 2020. Experts in otolith ageing and bomb radiocarbon methods discussed the encouraging results from preliminary radiocarbon work for tuna and agreed that the application of radiocarbon methods to validate age estimates derived from tuna and billfish otoliths from the WCPO was feasible. A design study was developed, using available otolith samples from the WCPFC Tuna Tissue Bank and other collections, and a draft research plan is proposed for consideration at SC16 (Appendix C). The recommendations from the workshop include that the:

- SC16 note the proposed research plan to continue the bomb radiocarbon age validation for bigeye tuna.
- SC16 note the proposed research plan for bomb radiocarbon age validation for yellowfin tuna.
- SC16 consider supporting these research plans with a budget contribution of USD97,980 (Appendix C)

Rational for workshop

Based on recent Western and Central Pacific Ocean (WCPO) bigeye tuna stock assessments (McKechnie et al., 2017; Vincent et al., 2018), the specification of growth in integrated stock assessment models such as MULTIFAN-CL can have profound effects on stock status indicators. It is therefore essential that such assessments utilise the best growth data and/or growth model estimates possible within such assessments. To this end, WCPFC in recent years has commissioned extensive research efforts to collect and analyse bigeye tuna otoliths (Farley et al., 2018), and more recently yellowfin tuna otoliths (Farley et al., 2020), to improve the assessments of those species in the WCPO. This work has relied mostly on counting presumed annual opaque zones in thin transverse otolith sections to provide the basis for determining annual age. Preliminary validation of this approach has been made through the analysis of several otoliths from tagged and recaptured tunas that had been injected with strontium chloride ($SrCl_2$) at release. At a recent workshop held at IATTC on bigeye and yellowfin tuna growth (IATTC, 2019), it was concluded that "Further direct age validation studies for bigeye and yellowfin daily and annual ageing methods, spanning the entire size range and expected range of longevity, are urgently needed in the Pacific."

During a follow-up technical workshop to compare ageing methods (Farley et al., 2019), recent progress was noted in the use of bomb radiocarbon methods (Ishihara et al., 2017; Andrews et al., 2020c) for the validation of tuna species, including Pacific bluefin, bigeye and yellowfin otolith-based annual ageing methods in the vicinity of Japan and the Gulf of Mexico. The report of the SC15-ISG-04 (*Review of SC14 BET Research Recommendations*) recommended to carry out further otolith age validation studies for bigeye and yellowfin in the WCPO such as applying radiocarbon age validation (short to long-term). As a first step to a potential age-validation study in the WCPO, it was proposed to hold an expert workshop to examine the feasibility and research design for such a project. This project was endorsed by SC15 and was approved at WCPFC16 to be conducted in 2020.

Due to Covid-19 travel restrictions, the workshop was held online on 1-2 July 2020 using Microsoft Teams. This report describes the workshop presentations, which are presented as short abstracts, and the important issues discussed by participants. The report is reasonably detailed in order to capture the points raised and discussed. We thank everyone who contributed to the workshop (see List of participants, Appendix A).

Opening and adoption of the agenda

Jessica Farley welcomed participants to the workshop and thanked everyone for joining. She gave some background to the workshop and noted that the goal was to determine the overall feasibility of applying the bomb radiocarbon method to the validation of opaque zone counts on bigeye and yellowfin tuna otoliths from the WCPO. We would then aim to specify a research design to undertake such a study. The aim would be to present the research plan to the WCPFC Scientific Committee meeting in August this year for funding consideration.

Jessica noted that the focus of the workshop would be mainly on bigeye and yellowfin tuna, as recent work has concentrated on those species. However, the workshop will also touch on other species, including swordfish and striped marlin as age and growth work is being undertaken on these species, but ages are not yet validated (see agenda, Appendix B).

Day 1 – session 1

Age estimation and validation of bigeye, yellowfin, swordfish and striped marlin in the Western and Central Pacific

Jessica Farley (CSIRO, Australia) presented an overview of the work undertaken by CSIRO, Fish Ageing Services, and SPC to estimate the age of bigeye tuna, yellowfin tuna, swordfish and striped marlin. The WCPFC Tissue Bank has been an important source of otoliths for the bigeye and yellowfin tuna work, and samples have been analysed from a broad area of the WCPO between 120ºE-130ºW and 10ºN-40ºS. CSIRO has collaborated with Fish Ageing Services on tuna ageing projects since the late-1990s, and FAS now undertake all otolith preparation and reading work. Using the data provided by FAS, we (until recently) calculated a decimal age for each fish using an algorithm that used counts of opaque zones, a nominal birth date (for all fish), capture date and the state of completion of the marginal increment (edge classification) of the otolith. However, a new age algorithm has been developed that uses only the counts of opaque zones and otoliths measurements to estimate decimal age. Age validation methods were described, including daily ageing to locate the first opaque zone, marginal increment analysis, and mark-recapture experiments. The presentation also described research to estimate the age and growth of swordfish and striped marlin and highlighted the lack of age validation available for those species worldwide.

The workshop noted that South Pacific albacore and skipjack tuna are other species that require further age validation, even though they are not a focus of this workshop. SPC noted that they had recovered nine sets of otoliths from tagged skipjack injected with strontium chloride that could possibly add to age validation of skipjack, in combination with other validation methods.

It was noted that the spatial and temporal distribution of otoliths in the ageing studies looks promising and that it may be possible to apply bomb radiocarbon dating, especially now being able to utilise the post-peak decline in the radiocarbon curve. Even with skipjack, if it had a six to eight-year longevity, the technique could be used to see if the birth years relative to the change in radiocarbon made sense over time, like the study of tunas in the Gulf of Mexico. At the very least, age scenarios could be eliminated, i.e. the fish are not older than a particular age, or they are much older than a particular age.

The workshop noted that a challenge with having otoliths collected from adult tunas is to determine their region of origin in order to match them to a radiocarbon reference. Given that tunas in the Pacific can move large distances at young ages, knowing the natal area is important for identifying which radiocarbon reference to use when matching the reference to the natal area.

It was suggested that SPC could concentrate on taking otoliths from recaptured tagged fish in the Pacific because, for these fish, the area in which they were tagged as young fish would be known. Many fish were tagged and released when they were around 40-50 cm, so they wouldn't necessarily be in their natal area (area of origin) but at least relatively close to the natal area. Although it's not something SPC has done traditionally, it might be possible to collect otoliths from a proportion of tagged fish, if they knew the areas and size classes to target.

The workshop noted that this is important because the bomb radiocarbon signal in some areas is consistent across broad regions but not in others. For example, the radiocarbon signal in areas north and south of the equator are different from the signal on the equator. A way of dealing with this is to use age-0 fish (young-of the-year, YOY) collected in a particular area covering a wide time span. It was noted that the WCPFC Tissue Bank has YOY otoliths from 2000 to 2020, a range coinciding with the decline period and, if those otoliths are from one area, it may be possible to see if there is consistency in change of radiocarbon over time. From this, you can relate the regional radiocarbon levels in early otolith growth to the core material of adult otoliths.

The workshop noted that otoliths from age-0 fish serve a similar purpose as obtaining otoliths from tagged fish because we can presume the natal area from location of capture. It was noted that bomb radiocarbon can be variable so the age-0 fish would be used to set up a baseline for radiocarbon in a particular area and specific time frame so that what you measure in the core of an adult is representative of that area and time frame.

The workshop asked if the bomb radiocarbon method was also applicable to elasmobranchs. Allen Andrews noted that unlike otolith micro-sampling, shark vertebrae can be analysed by serial sampling across the vertebrae from the apex to the tip. Most work has been on large-bodied sharks, from upper waters, although they do dive deep. A problem with some species is that vertebrae stop growing. Other confounding factors include signals from spending time at variable depths, in shallow and deep water, whether the vertebrae grow consistently over time, and that the source of carbon to the vertebrae is dietary compared to otoliths which are formed largely from dissolved inorganic carbon of the ambient seawater.

The workshop asked whether otolith elemental microchemistry could be used to identify broad natal areas in the western and central Pacific, including closer to and further from the equator, and what work has been done in this area? Jed Macdonald explained that for southern bluefin tuna and the Atlantic tunas, which have consistent seasonal spawning patterns, otolith microchemistry has been successfully applied to examine different spawning cohorts and trans-Atlantic movements into the Mediterranean. There have been a relatively few studies on tropical tunas, though some recent work on natal origins and connectivity in yellowfin, bigeye and albacore across the WCPO and Hawaii was noted (e.g. Rooker et al. 2016). An issue limiting otolith chemistry applications for certain tropical tunas is that they are able spawn often and over broad regions across which environmental gradients typically do not vary that strongly. Hence, defining what constitutes a local or regional natal chemical 'signature' can be challenging. Innovations in genetic techniques and particle simulators can provide additional and/or complimentary information on the scales of connectivity among populations, in addition to inference on stock structure and fidelity to spawning regions SPC in collaboration with CSIRO has recently commenced work on a design study to define the analytical tools (including otolith chemistry, genetics and simulation models), sampling coverage and investment required to resolve longstanding stock structure questions for these species.

Distribution and migration of bigeye, yellowfin, swordfish and striped marlin in the Western and Central Pacific

John Hampton (SPC, New Caledonia) presented a review of both data- and model-based evidence for the distribution and movement of bigeye tuna, yellowfin tuna, swordfish and striped marlin in the Pacific.

Bigeye tuna: Juvenile bigeye purse seine catches are virtually continuous across the equatorial zone of the entire Pacific, indicating an uninterrupted distribution. Longline catch per unit effort (CPUE) for bigeye tuna shows concentrations in the central – eastern equatorial Pacific and in North Pacific temperate waters. Tagging data, mainly for juvenile bigeye tuna, show extensive west to east movement in the equatorial zone, with limited exchange with the North Pacific area. SEAPODYM modelling reflects a distribution of adult biomass similar to that of longline CPUE. The available evidence indicates that bigeye tuna may be more locally resident nearer to the large islands and land masses in the far western and eastern Pacific, with considerable movement in the oceanic areas of the central Pacific.

Yellowfin tuna: Purse seine catches of primarily juvenile yellowfin tuna show concentrations in the western and eastern tropical Pacific, with a discontinuity in the central Pacific. In contrast to bigeye tuna, longline CPUE and SEAPODYM modelling indicates that the adult portion of the yellowfin tuna stock is strongly concentrated in the tropical waters of the western Pacific. Tagging data, mainly for juvenile yellowfin tuna, indicate mixing in the western Pacific, with a small number of observations of movement into the central and eastern tropical Pacific. There are some observations of movements from the tropical western Pacific into the north-western Pacific, Hawaii and South Pacific off Australia. However, there appears to be a degree of local residency around the archipelagic areas and large land masses in the western Pacific.

Swordfish: Swordfish catches are concentrated in the south-western Pacific and in the Pacific east of about 160^{o}W in the north and 130^{o}W in the south. Conventional and electronic tagging of swordfish released in Australia and New Zealand indicate some degree of separateness around about 170^{o}E longitude with considerable N-S movement within these areas. NZ releases range widely into the central Pacific to at least 150W.

Striped marlin: Striped marlin longline catch is concentrated in the south-western Pacific in the Coral/Tasman Seas and in the tropical Pacific east of 160^{o}W. Conventional tagging in New Zealand shows movements widely to the west and north-west into the Coral and Tasman Seas, north and east towards the Pacific Islands and French Polynesia. Pop-up satellite archival tagging from New Zealand shows individual tracks ranging widely to the north (New Caledonia, Fiji, Tonga) and north-east (French Polynesia and beyond). Conventional tagging off Australia indicate considerable north-south mixing, but with limited dispersal to the east.

The workshop discussed that the bomb radiocarbon signal in individuals is not just dependent on the origin of the fish, depth can complicate the bomb radiocarbon signal by becoming more depleted. This could affect the bomb radiocarbon signal in species like bigeye tuna, striped marlin and swordfish, depending on the depth at which the otolith material was formed. Yellowfin may not be complicated in this manner because they are documented to start life in the warmest surface waters and tend to remain in the mixed layer through ontogeny; hence, even if you catch an adult in areas with cooler surface waters, the date of formation can still be traced back to the area of the radiocarbon reference curve.

The workshop asked about the behaviour of some of the species that inhabit depths towards the lower end of the mixed layer and how that could affect the bomb radiocarbon method. John Hampton noted that there is information on bigeye and yellowfin (and probably on swordfish and striped marlin) on the depth behaviour of fish from archival tags. Bigeye occupy deep waters during daytime, below the mixed layer, and make some deep dives. There isn't lot of information about where they spend their formative months or years and at what depths. Animals need to be of a certain size to be able to effectively carry or withstand the surgery involved with archival tag deployment, so few archival tags are deployed on bigeye or yellowfin less than about 60 cm. The more archival tag data obtained, the more surprised we are about the depths these fish occupy. For skipjack tuna, a couple of archival tags have indicated they dive periodically to depths greater than 500 or 600 meters. They have been previously thought of as a creature of the surface layers, but the archival tag data indicates they can occupy deeper waters. Japan has carried out midwater trawl surveys, the results of which may be able to give us a better idea of where some of these small size classes of fish occur.

Allen Andrews added that, in papers by Brill et al. (2005) and Brill and Lutcavage (2001), Atlantic bigeye tuna, even young bigeye, were shown to make deep forays. He asked how can we gather information for bigeye and other species in this study?

John Hampton responded by referring to two additional papers, reporting on depth distributions of bigeye. The first examined archival data. The second modelled depth behaviour of bigeye tuna in relation to environmental characteristics, giving the means to predict bigeye distribution according to the temperature profile, thermocline depth and the size, which proved to be a significant feature of that model.

The workshop asked that if YOY otoliths were used to create the decline in the ^{14}C record, and if those fish had spent time at depth, could their average bomb radiocarbon levels still be used to compare with adults? Allen Andrews replied that with known collection date, material that has a slightly depleted signal compared to surface dissolved inorganic carbon (DIC) or regional coral records, it could be related to a depth depletion. With the bigeye tuna from the Gulf of Mexico there was one fish that was aged consistently to 12 years old, which was slightly lower than the rest of the fish in that group. Either it was from a different location (Gulf of Guinea on eastern side of Atlantic) or there was a depth-related depletion for this individual. More samples could confirm if depth depletion was a factor.

The workshop noted a paper by Grammar et al. (2015) that investigated bomb radiocarbon transport in the South Pacific Ocean. It reports on radiocarbon depletion at different depths for several fish species (not tunas). A report by Scutt Phillips et al. (2019) was also noted as it reports on depth distribution of three tunas, from information collected from acoustics at FADs. The study reports on the differences during day, night and predawn, and on the variability, adaptability and flexibility in depth.

The workshop asked how much of the otolith core material was sampled for analysing the bomb radiocarbon signal. Does the material come from the larval stage of life (giving a larval signal) or is more recently deposited material incorporated in the sample, close to 3 months of life? Allen Andrews noted that for the recent yellowfin and bigeye tuna work in the Gulf of Mexico (Andrews et al., 2020c), material was extracted from the cores that contained approximately 60 to 90 days' worth of material. This estimate was supported with daily increment observations in some cores where the increments were clear. The amount of material needed for analysis is about a milligram for a ^{14}C measurement with reasonable precision with accelerator mass spectrometry (AMS). "Gas-AMS" (at the Ion Beam Physics Lab of ETH Zürich) can analyse smaller sample masses of 300-500 micrograms of material because of increased instrument sensitivity — the advanced Mini Carbon Dating System (MICADAS) by Ionplus (https://www.ionplus.ch/micadas) — and the approach skips the graphitization step, which loses a considerable amount of material. This could then use only the otolith material closer to the core; however, with smaller samples the natural variability of the ambient marine environment becomes more apparent, making pinpointing the central value more difficult. A larger number of samples will strengthen the result by providing more confidence that the central value in the distribution is accurate for a given period of formation (i.e., along the monotonic radiocarbon decline period that would be used as a regional reference).

The workshop asked if having YOY fish at 3 months would still provide useful samples (rather than younger fish). Allen Andrews confirmed that having YOY fish at 3 months could be instrumental in a study. The "pie in the sky" would be to have fish that are YOY sampled from the 2000s back to the mid-1980s, just as the decline started to happen in most tropical locations of the Pacific Ocean. Radiocarbon measurements from otolith cores extracted from adults collected from the same regions could then compared with the juvenile radiocarbon reference.

The workshop asked about the distribution of bigeye in the eastern tropical Pacific. It appeared they straddled the equator, whereas the larval distribution was on either side of the equator with a gap along the equator. This is similar to the bimodal distribution of bomb radiocarbon across the equator, with elevated levels on either side of the equator due to different sources and mixing of the equatorial currents. Radiocarbon distribution is complicated, as is the bigeye distribution.

Allen Andrews noted that a regional radiocarbon signal relative to coral and DIC records could be identified in larval otoliths and be traceable in the earliest growth of the otoliths collected from juveniles and adults. Another technique to explore this is laser ablation (LA) accelerator mass spectrometry (AMS), where a scan would be made from the core along the otolith to analyse the otolith throughout a fish's life. This would likely show areas of depletion in the older fish because adult bigeye appeared to be associated with the South Equatorial Counter Current (SECC). Radiocarbon records from coral of the Galapagos Islands are some of the lowest and most variable records in the Pacific Ocean and may reflect adult portions of bigeye otoliths. This would be an interesting pursuit with LA-AMS to investigate radiocarbon patterns throughout the life of the fish, as was the case with red snapper in the Gulf of Mexico where the entire bomb radiocarbon signal was traced in individual otoliths (Andrews et al., 2019), and bigeye tuna would be a good candidate for pelagic fishes.

Day 1 – session 2

Introduction to bomb radiocarbon age validation and available ^{14}C records in the Pacific.

Allen Andrews (University of Hawaii) presented an overview of the bomb radiocarbon age validation method and its recent application to tuna species. Bomb radiocarbon dating is a method used to validate the age and growth of fishes that has been in use for nearly 30 years since its introduction in 1993 by Dr. John Kalish (a participant at the workshop). The method has expanded considerably with the discovery of more coral radiocarbon records that can be used in regional fish life history studies. The onaga (*Etelis coruscans*) is an Indo-Pacific snapper species that has provided an excellent example of how the method can work — ages to 55 years that were determined from growth zone counting in otoliths were validated with bomb radiocarbon dating in the Hawaiian Islands (Andrews 2020a). The method works by using the regional bomb-produced radiocarbon signal created by thermonuclear testing in the 1950s and 1960s that is stored in otoliths and corals as a time-specific marker. Further, the post-peak decline of the bomb radiocarbon signal has been shown to provide the opportunity to age fish that are not long-lived and where specimens are not available for birth years in the radiocarbon rise period (~1958-1970). Another good example of this application is with ulua or giant trevally (*Caranx ignobilis*) where fish collected in the 2000s were validated as living up to 25 years by relation to the radiocarbon decline in Hawaii (Andrews 2020b). At present, numerous species around the world have been validated for age, growth and longevity and the propagation of this signal has been traced through the Indo-Pacific and into the South Pacific where it can be used to age pelagic fishes such as billfishes and tuna — the focus of this workshop. Allen extended a "thank you" to John Kalish for paving the way for validating fish age using the bomb radiocarbon signal in otoliths, with his pioneering work on southern bluefin tuna (Kalish et al. 1996).

The workshop asked about how to determine which limb of the ^{14}C record to project back to (the rise or decline). Allen Andrews noted that if no growth zone structure was available to base the birth year on, it would be ambiguous. But if there is good otolith structure (defined zone formation), there is confidence in projecting back to the birth year based on growth zone counts. The use of otolith mass or in some cases fish length (although length typically becomes decoupled with age, e.g. Andrews (2020a)) can inform which limb to place it on if you are working on a species that has difficult to interpret otoliths. Some deduction from other sources is also possible.

The workshop asked about the importance of obtaining correct coral core ages. Allen Andrews noted that with the Kure Atoll record, each of the growth bands were sampled four times for Sr:Ca ratios as a proxy for seasonal temperature changes. Given that annual temperature changes in the environment correlated with Sr:Ca in the growth bands, the band counting is considered validated as annual (Andrews et al., 2016b).

Some concerns were raised about the bomb radiocarbon method, particularly when validating species for short to medium longevity, and whether we define the approach as validation or corroboration for ageing methods. Weight of evidence is used as an approach for addressing uncertainties in other applications. It was noted that validation is usually about validating the periodicity of increment formation in hard structures, rather than absolute ages. The strength of bomb radiocarbon is the corroboration, which for tunas and billfish would be welcome.

The workshop noted the variability in ^{14}C between coral records from American Samoa and the Cook Islands, which are relatively close spatially. The workshop asked why this might be the case? Allen noted that most of the records are pretty 'solid' based on the papers they were drawn from. The difference seen among areas is likely to be related to the gyre structure and residence times and potentially mixing of upwelled waters. American Samoa is more in the south equatorial current, whereas the Cook Islands and Easter Island are in gyre waters, so you would expect the locations in, or more toward the gyre, to be more elevated than those in equatorial currents.

The workshop noted that most of the coral core records stop in the late 1990s as that was when the coral samples were collected. To get more recent records we need to do a collection trip for more coral records, but some corals collected more recently from places like American Samoa (USGS) provide an opportunity to address the questions.

The workshop asked whether a regional and/or local ^{14}C reference for the decline period can be obtained based solely on YOY tuna samples that are available. Allen noted that YOY samples would represent a best-case scenario, as focussing solely on coral records means making a guess as to where the fish are relative to these records, and how widely variable the coral ^{14}C trajectories are, although the overall record of the decline period appears to dovetail as we approach the year 2020 — a composite radiocarbon record was shown that reflects this observation and was used to age blue marlin in the Pacific Ocean (Andrews et al., 2018). With the WCPFC/SPC otolith collections from 2000 to 2020, it may be possible to fill in gaps and possibly confirm the slope of the decline for the region, with secondary validation from coral records. This would provide a baseline reference to then look at cores of adults (if there are enough of them) even if they are highly mobile. The baseline work needs to be done first, then look at some adults and, as mentioned previously, perhaps use some of the tag recovery fish to isolate locations are bit more accurately.

The workshop noted that as analytical methods improve, smaller samples of otolith material are required. The workshop asked if the spatial scale problems discussed previously can be alleviated by doing time series of ^{14}C measurements, rather than taking an integrated chunk from the core of the otolith? Allen noted that the use of radial micromilling and LA-AMS is starting to increase. Error is still large, and a large amount of material is needed to get good results. Gas AMS goes one step further. A couple of hundred micrograms is enough to get a good ^{14}C value. These techniques are at the cutting edge and is likely to be the future of ^{14}C analysis for looking at within-individual variation of the radiocarbon signal, particularly in species with small otoliths. It was noted that when running LA-AMS, there is a need to stay in the same spot and acquire counts for some time. A 'zig-zag' scanning approach worked well on red snapper otoliths (which were large and easy to analyse), with the ability to gain sufficient material in the core region, for example (Andrews et al., 2019).

The workshop noted that the LA-AMS approach has advantages over using a milling approach, but with the downside of losing precision. The workshop noted that since bigeye and yellowfin exhibit fast growth, a 25-30 cm fish can be 50-60 days old, so it may be possible to use the whole otolith for the analysis, or the majority of it.

Use of post-bomb radiocarbon dating to validate estimated ages of Pacific bluefin tuna, *Thunnus orientalis*, of the North Pacific Ocean

Taiki Ishihara (National Research Institute of Far Seas Fisheries, NRIFSF, Japan) presented an overview of the recent bomb radiocarbon age validation study for Pacific bluefin tuna. Abstract from Ishihara et al. (2017): "*Estimation of the age of Pacific bluefin tuna (PBF), Thunnus orientalis, is one of the most important processes involved in assessment of its stock biomass. Recently, the ages of PBF have been estimated by analyzing otolith thin sections. In this study, the estimated ages of PBF were validated by use of post-bomb radiocarbon dating. The $\Delta^{14}C$ of the otolith core portion was measured by accelerator mass spectrometry and compared with reference values of corals in the western Pacific. The decrease of the $\Delta^{14}C$ of the whole otoliths of PBF that were 0 to 2-years of age from 1982 to 2010 coincided with the decrease of $\Delta^{14}C$ reference values. This result suggests that post-bomb radiocarbon dating can be used to estimate ages of adult PBF. The $\Delta^{14}C$ of the otolith core portions of fish estimated to be 2 to 27-years of age gradually declined as birth year approached the present day. This trend was consistent with the trend of the reference values and 10% lower. The portions of the otolith formed after 2-years of age remained on the surface of the adult otolith core; these portions would dilute the $\Delta^{14}C$ values of the otolith cores of adults and provide an explanation for the depletion. These results support the use of otolith thin sections to estimate PBF ages*."

Allen Andrews noted the vertical adjustment of ^{14}C values presented by Ishihara was valid and he supported the validity of the age estimates for Pacific bluefin tuna in the study.

Pacific blue marlin (*Makaira nigricans*) longevity estimates confirmed with bomb radiocarbon dating

Allen Andrews (University of Hawaii) presented the results of a recent study verifying the age of a large blue marlin (*Makaira nigricans*) captured in the Pacific. Abstract from Andrews et al. (2018): "*Longevity of blue marlin (Makaira nigricans) remains unresolved. Use of fin spines and sagittal otoliths for age reading have led to unconfirmed longevity estimates near 20–30 years. Age validation has been elusive because large individuals are rare and a technique that can be applied to structures that provide estimates of age was absent. Use of otolith chemical signatures has been limited by sagittal otoliths that are very small—whole otolith mass of adult blue marlin rarely exceeds 10 mg for the largest fish. Recent advances in the detection limits of radiocarbon (^{14}C) with accelerator mass spectrometry—coupled with recently acquired knowledge of marine bomb ^{14}C signals spanning the tropical Pacific Ocean—have led to an opportunity to age blue marlin from small amounts of otolith material. In this study, otoliths from a recently collected 1245 lb. (565 kg) female blue marlin at a measured 146-inch (371 cm) lower jaw fork length (LJFL) were analyzed for ^{14}C. Estimated longevity was either 12–21 years or 32–44 years based on bomb ^{14}C dating. Using multiple lines of evidence, it was determined that the young age scenario was most likely, with evidence for an age close to 20 years using a series of deductions in the bomb ^{14}C dating method*."

Because Allen previously mentioned new technology (see Andrews et al., 2019), the workshop asked how the LA-AMS data were aggregated along the transect. Allen noted that the post-processing of LA-AMS data was still work in progress. Allen reported that in previous red snapper work, an attempt was made to centre the bins to provide the most detail possible to highlight the initial rise of the bomb radiocarbon signal (~1958). In the future, we´ll be able to get better temporal and spatial resolution.

The workshop asked whether you would expect to see a difference in ^{14}C among the three sets of otoliths taken from the same fish? Allen noted that he has previously investigated this on the otoliths from a blue marlin to see if the lapillus and sagittal integration of ^{14}C over time was consistent and found that the measured ^{14}C values for both structures were nearly identical indicating that mass accretion rate between otolith types was quite similar. The workshop noted that the mechanisms driving ^{14}C uptake into otolith aragonite should be the same amongst the three types of otoliths, given that they are aragonitic. However, the workshop asked about another polymorph of $CaCO_3$ (e.g. vaterite or calcite) and whether there would there be any differences in uptake rates? Allen noted that maybe there would be difference in terms of the fractionation of ^{13}C relative to ^{14}C, but he thought ^{14}C levels would be independent of that, and everything is normalised relative to ^{13}C anyway.

The workshop asked about the coral reference records and whether the data is typically from just a single coral core analysed from a particular region, or are there multiple cores analysed, and would these likely show differences? Allen noted that it is typically just a single core extraction. There are a few spots with multiple records – e.g. Guadalcanal. The workshop noted that it would be interesting to see a comparison of corals collected side by side to gauge the variability, if any.

The workshop asked about the necessity for having YOY tropical tunas for the reference, or could you also use other plankton samples or larval fish, as the signals should be the same as for tunas. Allen noted that other pelagic species could indeed be used as a regional reference (e.g., short-lived species like sardines and anchovies). This is something that Allen is keen to do in Sweden where long time series of small fish collections exist, back to the 1930s (e.g. in the Baltic Sea).

Day 2 – session 1

Age validation of yellowfin (*Thunnus albacares*) and bigeye (*Thunnus obesus*) tuna of the northwestern Atlantic Ocean

Allen Andrews (University of Hawaii) presented the bomb radiocarbon approach applied to bigeye and yellowfin tuna in the Gulf of Mexico. Abstract from Andrews et al. (2020c): "*The age and growth of yellowfin (Thunnus albacares) and bigeye (T. obesus) tuna remain problematic because validation of growth zone deposition (opaque and transparent) hasn't been properly evaluated. Otolith growth structure (zone clarity) can be poorly defined for tropical tunas but the use of bomb radiocarbon dating has validated age estimates to 16–18 years for yellowfin and bigeye tuna. Use of the ^{14}C decline period — defined by regional coral and otoliths — provided valid ages through ontogeny. Yellowfin tuna aged 2–18 years (n = 34, 1029–1810 mm FL) and bigeye tuna aged 3–17 years (n = 12, 1280–1750 mm FL) led to birth years that were coincident with the bomb ^{14}C decline. The results indicate there was no age reading bias for yellowfin tuna and that age estimates of previous studies were likely underestimated for both species*."

The workshop asked about the measurement error on the ^{14}C data noting that they appeared to be quite small. Allen noted that they were quite tight as the sample of otolith that is required using the newer method is much smaller. For the LA-AMS work, the error bars are much higher but for that method it is more about replication of the procedure over time and looking for changes in radiocarbon through the entire structure (e.g., red snapper study; Andrews et al., 2019).

The workshop asked whether the samples were collected in similar areas and whether the collection area has an influence on the results. Allen noted that the samples used in the analysis were all thought to be of western Atlantic origin. They are unlikely to be of Gulf of Guinea origin; however, it should not be ruled out entirely. The workshop noted that the situation might be the same for Pacific samples, where the origin (east or west) can be inferred but is not absolute.

The workshop asked about the method used for the preparation of the YOY samples and methods to ensure that the main otolith material that corresponds to the first 6 months of growth is collected. Allen noted that the method is based on a modified method that cuts a thick transverse section. The lobe on the top of the otoliths is manually removed with a scalpel under a dissecting microscope. Alternate methods to obtain core material were discussed by participants. For example, for the early southern bluefin tuna study, a Dremel tool was used to sculpture the otoliths by hand to isolate the otolith core and remove the ancillary growth areas that would be composed of more recently formed material. This is a fiddly method; however, a technician can become proficient relatively quickly.

The workshop asked about the outliers in the data and how to determine if the error is likely to be associated with ageing error or the measurement error and whether Allen looked at otolith weight? Allen noted that otolith outliers can be re-examined to confirm the age estimate. In the Gulf of Mexico study, some were re-aged and found to be a slightly different age compared to the originally assigned age, while others were re-aged several times and the age estimate proved to be consistent.

The workshop noted one of the bigeye that was an outlier showed ^{14}C that was more depleted than the reference line; this could have been a sample where the more recently formed material was incorporated into the analysis. Allen noted that the new material would need to account for approximately 40% of the material, therefore, a more likely scenario is that this sample may have had a different origin to the others or was an example of ^{14}C depletion due to depth-related factors.

The workshop asked about the specific costs and whether it would be feasible to be able to provide an absolute age from a large number of samples using this method. Allen noted that the high cost (~$300-600/sample, depending on facility and funding sources). Methods are being trialled/developed to try to reduce costs (i.e., multiple staging of otolith samples for gas-AMS or LA-AMS).

The workshop recommended that the Gulf of Mexico yellowfin age-at-length relationship figure (presented as part of Ashley Paccio's full yellowfin age and growth thesis) should have the ^{14}C validated samples highlighted by colour or labelling. The workshop agreed and recommended that this should be done for any age-at-length plots produced for the Pacific fishery samples. The workshop also noted that the variability in length-at-age of yellowfin in the Gulf of Mexico study, and that this was also discussed at the yellowfin and bigeye ageing workshop held at the NOAA Panama City Laboratory (Dec 2019). Several of those outliers were checked during that workshop and that for most cases the assigned aged was confirmed as being a reasonable estimate.

The workshop asked about whether the ^{14}C reference data, particularly those obtained from other fish species, could be done for the Pacific reference curves. Allen suggested that it is possible to use age-0 otoliths from other fish species to help develop Pacific reference curves; however, it would be better to get reference curves from coral records, especially if it is possible to get coral samples from known origin areas.

Preliminary results of bomb radiocarbon analysis for the WCPO-BET otolith

Kei Okamoto (NRIFSF, Japan) presented preliminary findings of ^{14}C isotope analysis for age-0 bigeye tuna in the WCPO. The radiocarbon analysis has not been applied to WCPO bigeye tuna before. Okamoto analysed 29 age-0 juveniles (FL: 26.9 – 46.2 cm) to obtain a preliminary reference dataset for the decline in the radiocarbon curve. The age-0 specimens were caught in 2010 to 2018, except for 2011 and 2016, in the WCPO tropical waters ranged from 4.0°N to 7.0°S in latitude and 144.0°E to 164.0°E in longitude. A scatter plot of the $\Delta^{14}C$ value and catch date show decreasing trend from the past to the present. The $\Delta^{14}C$ value from otoliths showed a good fit to the reference Kure Atoll coral records (loess fit). Okamoto concluded that age validation of larger fish would be possible in the future using the results of this study if supplemented with analysis if additional age-0 BET otoliths (e.g. from the WCPFC Tissue Bank) to improve reference dataset.

The workshop asked about the decline relationship in ^{14}C values in age-0 bigeye and whether there was any latitudinal pattern detected between those samples with higher or lower values when compared to the reference line. It was suggested that it could be influenced by different oceanographic effects, like ocean current sources. Okamoto-san noted that this had not been investigated but suggested that as some samples were missing the otolith tips and since the whole YOY otolith was used, perhaps the variability in values was due to this rather than location of capture.

The workshop asked whether consideration had been given to using a better coral core reference, one that is closer to the area of capture than the Kure Atoll. The workshop suggested that perhaps Palau, Guam and possibly Paluma coral records could be used as a good equatorial signal, and also the Paluma coral reference. Allen Andrews volunteered to assist with making an initial analysis of the data with respect to other coral records (and to provide those data to Okamoto-san) and possible latitudinal patterns in the otolith ^{14}C levels across the decline period.

Draft workplan of radiocarbon analysis for bigeye tuna in the WCPO

Kei Okamoto presented draft candidate specimens for future radiocarbon analyses of WCPCO bigeye tuna. The suggested workplan had two components, which were (i) to improve the Δ^{14}C slope model using age-0 samples and (ii) compare core ^{14}C values from otoliths read for annual age to the Δ^{14}C slope model. First, regarding the improvement of Δ^{14}C slope model, Okamoto indicated there no samples were analysed before 2010, or from 2011 or 2016. Thus, he suggested that analysing specimens caught in those years would improve of Δ^{14}C curve and confirm whether otoliths showed a good fit to the reference Kure Atoll coral records. Okamoto suggested that approximately 100 specimens would be sufficient, and he presented the catch locations of candidate age-0 bigeye tuna, which were smaller than 50 cm FL. Next, a suggested workplan for the age validation for specimens already aged (annual age) was presented. It was suggested that approximately 100 'sister' otoliths of those previously read by Fish Ageing Services would be analyzed. These would be from a wide range of years and caught in WCPO tropical waters adjacent with the localities of the age-0 specimens analyzed. This assumes there is relatively restricted movement from natal areas.

The workshop noted that a strong El Niño (2015-16) was observed during the period in which juvenile bigeye tuna otolith were sampled from the western Pacific Ocean, and that the resulting shallower thermocline may explain some of the variation in ^{14}C observed in the otoliths. However, the workshop also noted that bigeye tuna may adjust their position in the water column to remain primarily in their preferred habitat, which may reduce variation in otolith ^{14}C levels.

The workshop noted that the bimodal distribution of larval bigeye tuna, in the warmer shallower waters either side of the equator in the eastern Pacific Ocean, would provide a good record of ^{14}C in the otoliths which could be correlated with ^{14}C levels from coral records in the region. The workshop also agreed that the application of LA-AMS to adult otoliths would be useful to identify how ^{14}C levels change through the life of the fish, which may corroborate the movement patterns from tagging data.

The workshop noted that while bigeye tuna tend to be resident in the far eastern Pacific Ocean, and to some extent in the far western Pacific, it is not the case in the central Pacific, where there is a strong tendency for fish to move towards the east. This behaviour should be considered in the sampling design.

The workshop discussed at length the relative merits of using coral cores versus otoliths for developing the ^{14}C reference curves for different regions. Coral cores were considered advantageous as they represent a static (in space) reference point and would allow us to extend the current time series available from otoliths. It is possible that several coral core samples available from AIMS and ANU could be analysed without the need to collect more cores from the field. However, the workshop considered that additional coral cores would be needed from specific regions, which would be logistically difficult and expensive to collect and analyse. Furthermore, the workshop agreed that otoliths from age-0 bigeye tuna would provide a better indication of the ^{14}C signature for the bigeye tuna habitat than would coral cores and would provide a better measure of the variability in the ^{14}C otolith signature over time.

The workshop discussed the merits of using archived bigeye tuna otoliths from previous SPC and CSIRO studies in the early 2000's to add to the ^{14}C reference curve. These otoliths have been sectioned, so they would only be useful if the sister otolith is still available or if there is sufficient material remaining from the sectioned otolith to sample core (early growth) material. Alternatively, LA AMS could be used to sample a series of spot measurements from multiple otoliths, which could reduce the amount of material needed from each individual otolith and reduce the overall cost.

The workshop acknowledged that often otoliths were used for multiple analyses, including ageing, microchemistry and bomb radiocarbon, and that care would need to be taken in sample preparation to conserve sufficient material for multiple analyses. The workshop also noted that the otolith core was not always needed for bomb radiocarbon analysis, and that the rostral tip from the otoliths of age-0 individuals could also be used. However, for age-0 fish, the whole otolith is typically embedded when sectioning for age estimation, so the rostral tips would be difficult to retrieve.

Day 2 – session 2

Availability of samples from the WCPFC Tissue Bank

The original draft workshop agenda included the presentation of a draft workplan of radiocarbon analysis for yellowfin tuna (and billfish) in the WCPO would be presented. As this had not been developed, a preliminary summary of age-0 otoliths for bigeye, yellowfin and albacore tuna in the WCPFC Tissue Bank was presented. The number of otoliths collected by year indicated that there are gaps in otolith collections for some years, in particular, 2007, 2008, 2010 and 2012. The largest number of otoliths were from the exclusive economic zones (EEZ) of Papua New Guinea, the Philippines and Solomon Islands. The sampling locations of the otoliths included in the bigeye and yellowfin tuna ageing studies was also provided as a reminder, as well as histograms of the back-calculated birthyears of these specimens.

Discussion on feasibility of the approach

The workshop agreed that a follow-up meeting was required to discuss the availability of samples from the WCPFC Tissue Bank, and to determine the criteria for selecting otoliths for analysis. This should include obtaining information on the quality/condition of samples (e.g. broken, sectioned) and availability of sister otoliths to ensure there are sufficient samples for the analytical approach. In selecting otoliths to analyse, it will be important to consider the amount of material sampled from the otolith and the time period over which this sample represents.

The workshop noted that the ^{14}C reference curve has been converging among different locations over the past 20 years. This means that it may not be an issue to compare recent (post ~2000) collections of whole otoliths from juveniles from different locations with the otolith cores from adults from different locations. The workshop agreed that a comparison of otoliths from age-0 individuals across a broad area with the coral ^{14}C reference curves would provide some confidence in using age-0 otoliths for a reference curve.

The workshop agreed that an initial selection of otoliths from the WCPFC Tissue Bank for the reference curve should focus on the past 20 years (2000-2020) with replicate age-0 fish in each year from a representative northern (n=2) and southern (n=2) location (and perhaps east and west locations). The workshop also agreed that the selection of adult fish for age validation should focus on a more recent single year (e.g. 2017) and select otoliths from a range of age classes. This approach would allow the matching of the decline record from adult otolith cores with that from the juveniles; hence, the oldest fish would be expected to have the most elevated ^{14}C levels and the youngest fish the lowest levels (oldest to youngest fish would trace monotonic radiocarbon decline represented by the 0-age reference series) — this approach was used in the tuna study of the Gulf of Mexico (see sample selection years for yellowfin tuna in Andrews et al., 2020c).

The workshop noted that Okamoto-san has already started the work on developing a sampling strategy for bigeye tuna and agreed to work in the inter-sessional to determine if any value could be added to that existing sampling design for bigeye tuna. Allen Andrews offered to share the core extraction method used in the Gulf of Mexico study.

The workshop noted that there were two aspects that could be explored with the LA-AMS: i) the efficiency gained by laying out a series of otolith cores or age-0 otoliths for serial sampling, as opposed to analysing individual samples (Allen Andrews is collaborating with ETH Zürich in Switzerland on this new application of LA-AMS technology), and ii) analysing transects across whole otoliths from adult bigeye or yellowfin tuna to examine how the ^{14}C changes over time within individuals — selecting the largest (oldest) fish would be most appropriate for this analysis.

The workshop noted that for the validation/corroboration of age estimates, it would be beneficial to use the existing otolith material (e.g. sister otolith), if available, from adult fish that have already been aged. This would reduce the need to age additional fish. The workshop agreed that locating these samples should be a priority.

The workshop noted that both otoliths from many of the young fish in the sample archive have been used for ageing, with one otolith sectioned and the other ground. However, there are still likely to sufficient numbers of sample remaining with sister otoliths available.

The workshop identified the need to consider the budget when deciding on the sample size required. A total of 80 age-0 fish would be needed to develop the reference curve if the sampling design consisted of 4 fish each year (2 from north, 2 from south) over a 20-year period. The workshop considered this to be the minimum sample size required to develop a reference curve. For the validation/corroboration, the workshop agreed that the best approach would be to select a recent year with the largest sample size of adult fish across the broadest estimated age range and select 1 or 2 fish from each age class.

The workshop agreed that it would be useful to see the results presented by Okamoto-san by latitude to see whether latitude should be considered when selecting age-0 otoliths for the bigeye tuna reference curve. The workshop agreed that this information should be presented at the follow-up meeting in 1 week.

The workshop agreed that if resources were limited, then yellowfin tuna should be a priority over bigeye tuna, since we already have some validation available for bigeye otoliths for the strontium chloride mark-recapture work in the Coral Sea.

The workshop noted that the small budget to hold an initial workshop has not been spent, due to travel restrictions, and may be available to commence some preliminary work on this project. Permission to use these funds will require permission from the SC and a contract variation.

The workshop agreed that a proposal will be submitted to the WCPFC Scientific Committee that will describe the sampling strategy and which samples will be required from the WCPFC Tissue Bank. This proposal may also describe what budget is required for the work. The workshop also noted that Japan will be seeking funding from the SC to progress the work on bigeye tuna.

The workshop acknowledged that the SC would ultimately decide on their preferred approach for this work to progress but agreed that the SC would likely prefer a single project to simplify project management.

The workshop noted the preliminary information extracted from the WCPFC Tissue Bank on yellowfin and bigeye juvenile otoliths, and that there were some years between 2000 and 2020 in which no age-0 yellowfin or bigeye tuna were sampled. The workshop also noted that sample sizes were very small across most EEZs, but larger sample sizes were available for yellowfin tuna from Papua New Guinea (PNG), Philippines, the Solomon Islands and some of the high seas areas, and for bigeye tuna from PNG, Philippines, Phoenix islands, Tokelau and Tuvalu.

The workshop noted that PNG and the Philippines were two locations with large samples for both yellowfin and bigeye tuna, and these could be two locations to focus on.

The workshop noted that skipjack otoliths could be used as additional material for the reference curve and to fill in any gaps in years or locations for yellowfin and bigeye tuna. However, the gaps in years appear to be similar across all species, so there may be limited utility for skipjack otoliths.

The workshop noted that there are some earlier collections of juvenile otoliths (from the 1990s) that could be used to extend the reference curve back in time. However, the workshop also noted that these samples may not be very useful if the adult otoliths that are to be validated/corroborated were not from fish spawned in these earlier years.

The workshop agreed that it would be useful to include a latitudinal component to the selection of age-0 otoliths for the reference curve, and that we could use otoliths from the Philippines (north), PNG (equatorial) and Solomon Islands (south).

The workshop noted that otolith weight would be useful information to use to obtain a wide range of ages across the otoliths selected for validation/corroboration.

The workshop noted that Allen Andrews will discuss with ETH Zürich the likely cost for conducting serial sampling of 80 juvenile otoliths in one or two batches, which can then be compared to the cost of analysing the samples individually.

The workshop agreed that an initial screening of the WCPFC Tissue Bank should focus on juvenile yellowfin and bigeye from 3 locations (Philippines, PNG and Solomon Islands), and extract information on the year sampled, otolith size, material that is available (e.g. sister otolith), and analyses that have been completed (e.g. sectioned and aged).

The workshop noted that juvenile samples collected from Indonesia by CSIRO are unlikely to be useful to fill in any gaps in years because these samples were collected in only two recent years (2013-14).

The workshop agreed that it would be useful to contact Chi-Lu Sun from Taiwan to see if he has any yellowfin tuna otolith samples from around the Philippines from his previous age and growth work.

The workshop noted that there may be a difference in the ^{14}C refence curve for bigeye and yellowfin tuna due to differences in vertical behaviour. However, archival tagging data suggest that juvenile bigeye tuna spend less time in deeper water than adults, so there may not be much difference in the reference curve between species. The workshop agreed that a smaller sample of age-0 bigeye tuna otoliths could be used, from similar locations as yellowfin tuna, to determine if there are any differences between species.

The workshop suggested that Japan and Allen Andrews discuss (and report back at the follow-up meeting in a week) whether the preliminary work done by Japan on bigeye tuna is sufficient for defining the reference curve, or whether additional samples and analyses (e.g. from other locations) would be beneficial.

The workshop noted that the choice of machine or laboratory does not significantly affect the analysis of ^{14}C because all laboratories use the same reference standards from 1950, and inter-laboratory comparisons have shown that the margin of error is well within the variability observed in the post-peak bomb ^{14}C decline.

Meeting close

Jessica Farley thanked the participants for participating in the e-workshop and closed the meeting.

Follow-up meeting (Monday 13 July)

It was agreed at the workshop that a follow-up meeting was required to report back on:

1) Whether there was a latitudinal pattern in bigeye age-0 ^{14}C values from Okamoto-san's preliminary work in the WCPO.

   Allen Andrews worked with Okamoto-san and Satoh-san to generate a plot of the ^{14}C values split into three latitude bands (>2N, 2N-2S, >2S), which indicated no clear pattern with latitude apart from slightly lower levels in otoliths from >2S. This might be expected for the southern hemisphere. The data was also separated by water masses and current structure across a section of the region of interest (140E-170E), which gave a slightly different distribution but again showed no clear latitude or current origin patterns. The results appear to indicate the natural variability in the system at a time where the ^{14}C values are starting to dovetail together across vast areas of the tropical Pacific — any future study will need to deal with this variability (~±10‰ $\Delta^{14}C$ on a monotonic decline of ~–2.5‰ per year) when the decline is used as a reference series. It was noted that the slope of the decline in ^{14}C was in line with the decline from numerous coral records. However, more data is needed to show if a latitudinal pattern exists or not. The workshop noted that there would be benefit in plotting the residuals of each individual measurement from the mean regression. That might help determine if there is a latitudinal trend, rather than grouping the individuals into latitude bands.

2) How the bigeye age-0 ^{14}C values from Okamoto-san's study compared with the regional coral records.

   A plot was shown (Figure 1) comparing the ^{14}C values from Okamoto-san's study on bigeye, Ishihara-san's data for Pacific bluefin (Ishihara et al. 2017) and the available coral reference records across the Pacific. The bigeye values fell in line with what is expected.

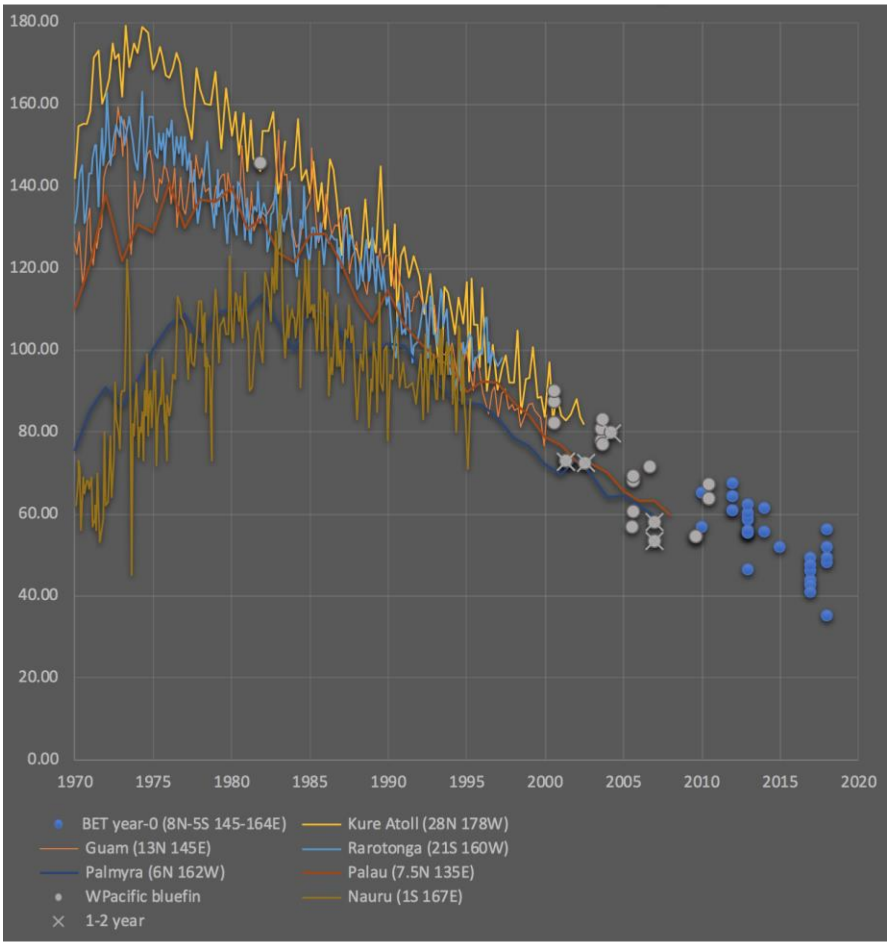

Figure 1. Plot of radiocarbon data from various coral records of the tropical Pacific Ocean with the otolith measurements made from two tuna age and growth studies: 1) Pacific bluefin tuna juveniles from the western central Pacific age-0 with 1-2 year highlighted with an X (Ishihara et al. 2017) and 2) preliminary bigeye tuna juveniles (age-0) from latitudes 8N to 5S across 145E to 164E (Okamoto and Satoh; Unpublished data). Note the consistency of the decline relation and that records are beginning to dovetail in the 2000s. Coral records: Kure Atoll (Andrews et al. 2016b); Rarotonga (Guilderson et al. 2000); Guam (Andrews et al. 2016a); Palmyra (Druffel-Rodriguez et al. 2013); Palau (Glynn et al. 2013); Nauru (Guilderson et al. 1998).

3) A proposed research plan for bomb radiocarbon age validation for yellowfin tuna.

   Allen Andrews presented a proposed research plan, which consisted of the following four elements:

   A. Process a series of 0-age yellowfin covering 20-year span: i) select 60 juveniles with replicate years; ii) explore potential use of N-S component and either describe or alleviate possible complications.

   B. Process a series of 20 adult otolith cores from 2-14 years and assess alignment vs. misalignment from estimated ages and calculated birth years. This establishes the feasibility of running more yellowfin adults now and through time, potentially tracing changes in population age structure. This also sets precedent for follow up studies (e.g., skipjack, albacore, billfishes).

   C. Explore use of gas- and LA-AMS for radiocarbon measurements: i) primary approach with gas-AMS because it is efficient and less expensive with much lower sample masses (~300-500 ug). This approach allows a focus on the first few months of growth at a cost that is 1/3 to 1/6 of normal per sample cost. LA-AMS may provide serial samples but this would need to be an exploratory assay as it has not been done on serial samples. Costing with ETH Zürich is not for routine analyses and via personal and professional arrangement with colleagues.

   D. Full study may take 6 months to 1 year to complete: i) sample selection, extraction, preparation and instrument use; 2) site visit (ETH) to perform assays and provide live analysis of data. End result is a transparent synthesis with likely publishable manuscript on ground-breaking study with ETH Zürich.

   To assist with consideration of the project details, a draft research plan will be proposed for consideration at SC16 The meeting discussed whether the otolith edge could be used in older fish rather than the core as the capture location may be known precisely at the time the edge material was deposited (i.e., the fish capture location). This assumes that there is no ontogenetic change in the uptake of ^{14}C. Allen noted that he had used the rostrum tip as temporal references as the material had formed in the last 6 months. In a parallel study, you could look at both parts of the otolith and Allen has done so with a snapper species in Guam. The meeting noted that this may help with the issue of whether a YOY fish had moved, or was diving to deeper levels, during the first year of like resulting in variable uptake of ^{14}C between individuals.

   The meeting asked whether the LA-AMS approach could be applied to otoliths already sectioned for age estimation, so that validation could be applied directly on the otolith being aged. Allen noted that one of the limitations is the spot size is fairly large (~70x120 microns). To get good measurements you need to stay in one spot for a while. The other limitation is the depth of the otolith section; it will be difficult to run the sample since you do not want to go through into the adhesive material, as it will be completely ^{14}C-depleted ,so you would get a downward spike in ^{14}C levels. This was observed for the red snapper study, but an otolith thickness of just 0.3 mm was used, and the method was successful in that case in revealing a full bomb-produced radiocarbon signal (see Andrews et al., 2019).

4) A proposed research plan for bomb radiocarbon age validation for bigeye tuna.

Okamoto-san provided a short summary of the proposed workplan for bigeye tuna that was provided on Day 2 of the workshop (see above). YOY otoliths have been identified in the WCPFC Tissue Bank for the years in which no samples have been analysed.

To assist with consideration of the project details, a draft research plan will be proposed for consideration at SC16.

5) The availability of suitable otoliths for ^{14}C analysis (age-0 and ‘sister’ otoliths of fish aged).

An update on the number of otoliths from age-0 bigeye, yellowfin and skipjack available in the WCPFC Tissue Bank was presented. It was noted that although large sample sizes were available for some locations (e.g., PNG, Philippines, and the Solomon Islands), the samples were not collected over a long time period, apart from those collected in PNG, although there were still gaps for some years. Further work is required to determine the criteria for selecting otoliths for analysis. Specifically, YOY otoliths to examine variability in ^{14}C among species, years and regions. Further work is also required to identify ‘sister’ otoliths of aged fish in the WCPFC Tissue Bank that are suitable for analysis. Currently, the Tissue Bank only has this information recorded for a subset of otoliths. However, preliminary investigation suggests that there should be ample sister otoliths for ^{14}C analysis in the Tissue Bank. In addition, it was recommended that otoliths from more older fish be located and aged due to the low number of maximum age fish (> age 10 years) and that high otolith mass could be used as a proxy for the oldest fish.

Recommendations

The workshop recommends that:

SC16 note the proposed research plan to continue the bomb radiocarbon age validation for bigeye tuna.

SC16 note the proposed research plan for commencing work on bomb radiocarbon age validation for yellowfin tuna.

SC16 consider supporting these research plans with a budget contribution of USD97,980 (Appendix C).

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Appendix A: List of participants

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Naomi Clear	CSIRO, AU
Paige Eveson	CSIRO, AU
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John Kalish	Department of Foreign Affairs and Trade, AU
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Hiroshi Minami	National Research Institute of Far Seas Fisheries, JP
Shuya Nakatsuka	National Research Institute of Far Seas Fisheries, JP
Kei Okamoto	National Research Institute of Far Seas Fisheries, JP
Keisuke Satoh	National Research Institute of Far Seas Fisheries, JP
Keith Bigelow	NOAA (Pacific Islands Fisheries Science Centre), US
Paul Hamer	The Pacific Community (SPC), NC
John Hampton	The Pacific Community (SPC), NC
Jed McDonald	The Pacific Community (SPC), NC
Simon Nicol	The Pacific Community (SPC), NC
Graham Pilling	The Pacific Community (SPC), NC
Mark Fitchett	Western Pacific Regional Fishery Management Council, US
Allen Andrews	University of Hawaii, US

Appendix B: Agenda

Bomb radiocarbon age validation workshop for tuna and billfish in the WCPO

Provisional Agenda

1-2 July 2020

All times are in Australian Eastern Standard Time (AEST)

Wednesday 1 July	**Workshop Items**	
15:00 - 15:30	Welcome and introduction Meeting procedure Goals Review of agenda	Farley
15:30 - 16:00	Age estimation and validation of BET, YFT, SWO and STM in the WCPO	Farley
16:00 - 16:30	Distribution and migration of BET, YFT, SWO, STM in WCPO	Hampton
16:30 - 17:00	*Break*	
17:00 - 17:30	Introduction to bomb radiocarbon age validation and available ^{14}C records in the Pacific.	Andrews
17:30 - 18:00	Use of post-bomb radiocarbon dating to validate estimated ages of Pacific bluefin tuna, *Thunnus orientalis*, of the North Pacific Ocean	Ishihara
18:00 -18:30	Pacific blue marlin (*Makaira nigricans*) longevity estimates confirmed with bomb radiocarbon dating	Andrews
	Close	

Thursday 2 July	**Workshop Items**	
15:00 - 15:30	Age validation of yellowfin (*Thunnus albacares*) and bigeye (*Thunnus obesus*) tuna of the northwestern Atlantic Ocean	Andrews
15:30 - 16:00	Preliminary results of bomb radiocarbon analysis for the WCPO-BET otolith	Okamoto
16:00 - 16:30	Draft workplan of C14 analysis for bigeye tuna in the WCPO	Okamoto
16:30 - 17:00	*Break*	
17:00 - 17:30	Availability of samples from the WCPFC Tissue Bank	MacDonald/Farley
17:30 - 18:30	Discussion on feasibility of the approach in the WCPO	
	Close	

Appendix C: Proposal

Project XX	**Bomb radiocarbon age validation for bigeye and yellowfin tunas in the WCPO.**
Objectives	To test the validity of age estimates for bigeye and yellowfin tuna from the western and central Pacific Ocean (WCPO) using bomb radiocarbon dating.
Rationale	As seen from the recent assessment of WCPO bigeye tuna (BET; McKechnie et al., 2017; Vincent et al., 2018), the specification of growth in integrated stock assessment models, such as MULTIFAN-CL, can have profound effects on stock status indicators. It is therefore essential that such assessments utilize the best growth data and/or growth model estimates possible within such assessments. To this end, WCPFC in recent years has commissioned extensive research efforts to collect and analyze BET (Farley et al., 2018; 2019; 2020a), and more recently yellowfin tuna (YFT; Farley et al., 2020a) otoliths to estimate growth to inform stock assessments. This work has relied mostly on counting presumed annual opaque zones in otolith sections to provide the basis for determining annual age. Limited age validation of the otolith reading approach was made through an analysis of several strontium chloride ($SrCl_2$) marked tuna otoliths that were tagged and recaptured. A recent workshop held at IATTC on BET and YFT growth (Farley et al. 2019) made the following conclusion: "Further direct age validation studies for bigeye and yellowfin daily and annual ageing methods, spanning the entire size range and expected range of longevity, are urgently needed in the Pacific." Recently, annual age reading protocols for YFT and BET in the Gulf of Mexico were validated using bomb ^{14}C dating (Andrews et al. 2020). The study used an innovative approach to the method where the post-peak bomb ^{14}C decline period (~1980–2000) was used to successfully validate YFT aged 2 to 18 years and BET 3 to 17 years. This new approach is well-suited to shorter lived species and was recently applied to Pacific bluefin tuna (PBT; Ishihara et al. 2017). This method relies on otolith ^{14}C levels in the core (earliest growth) as compared to formation years of a ^{14}C reference, often a validated coral core chronology, for the region of interest to determine if the calculated birth year from growth zone counts is consistent with the ^{14}C reference. At the most recent SPC pre-assessment workshop (April 2020), the bomb radiocarbon method was presented using BET 0+ aged fish (young-of-the-year) from the WCPO to investigate the distribution of ^{14}C in otoliths in time. Based on regional coral records and the results from PBT (see Figure 1 of Farley et al. 2020b; SC16-SA-IP-17), the approach looks promising for a full application of bomb radiocarbon dating to BET with an extension of its use to YFT. As a first step to a potential age-validation study in the WCPO, an expert workshop was held in July 2020 to examine the feasibility and research design for such a project (Farley et al. 2020b). During this workshop, Kai Okamoto (NRIFSF) presented the preliminary BET bomb radiocarbon results and proposed a draft workplan. As a follow-up to the workshop, Allen Andrews (University of Hawaii) presented a research plan proposal for bomb ^{14}C dating of YFT in the WCPO. These proposals have since been combined as a collaborative effort to increase efficiency and to take advantage of new ^{14}C accelerator mass spectrometry (AMS) technology at the Ion Beam Physics Lab of ETH Zürich, Switzerland.

<table>
<tr>
<td>Assumptions</td>
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- Otoliths identified as available by project partners are provided in timely manner.
- Otoliths provided by project partners, and those from the WCPFC Tuna Tissue Bank, are of sufficient quality to determine ^{14}C levels.
- Otoliths from the WCPFC Tuna Tissue Bank will be released without needing to have the research proposal approved by the SC Research Committee.
- Work to be completed by project partners is finished on time.
- Covid-19 travel restriction are lifted to allow travel to ETH Zürich, Switzerland.
- Allen Andrews and NRIFSF will undertake the core work and actively collaborate with CSIRO and the Scientific Services Provider in the conduct of the analyses.

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<tr>
<td>Scope</td>
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Otoliths of juvenile YFT and BET tuna collected through time from the WCPO will be used to establish a reference curve for bomb-produced ^{14}C that will provide a baseline for testing the validity of adult YFT and BET age and longevity estimates.

The reference curve will be composed of 0+ aged fish that were collected from fishing regions where both juveniles and adults have been collected over a 20-year period to reflect the post-peak bomb ^{14}C decline. Coral records that are proximal to the region indicate the reference record will be common across the latest 20 years (2000 to 2020; Figure 1 and see Figure 4 of Andrews et al. (2018)). The measurable monotonic decline variability with a strong central tendency will provide a basis for validation of age for adults using otolith cores (within the first year of growth). This approach is similar to the recent success demonstrated for YFT and BET in the northwestern Atlantic Ocean where ages approaching 20 years, along with an age reading protocol that is similar to what is now being used in parts of the Pacific Ocean, were validated for each species using regional coral and otolith reference materials (Andrews et al. 2020). The proposed study has the advantage of juvenile tuna otoliths that cover the entire 20-year reference period to be used in validating the estimated birth years of recently collected adults (i.e., 2015 has fish aged 1-14 years = birth years 2014-2001).

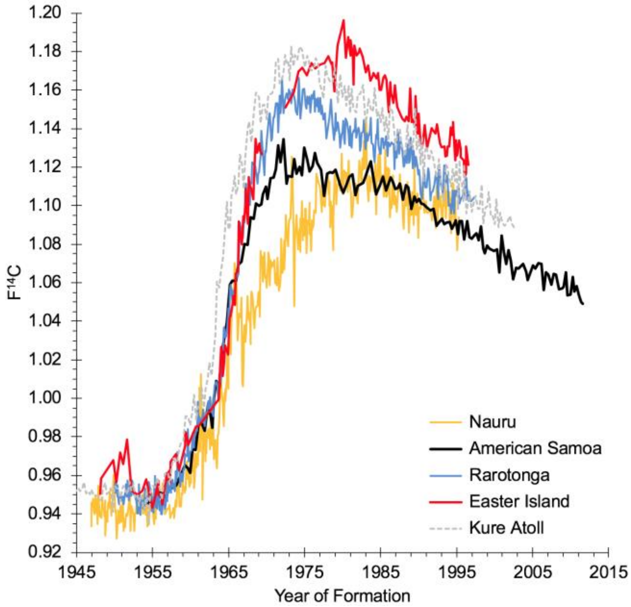

Figure 1. Cross section of coral radiocarbon records from north to south Pacific Ocean showing an apparent dovetailing of reference records in the most recent years (>2000) — this observation is consistent with air-sea diffusion of radio-CO_2 as the marine system becomes the bomb-produced radiocarbon reservoir (see Andrews et al. (2016) for first indication of crossover). The absence of other records to confirm the most recent years will be remedied

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with the 0+ aged tuna otoliths from the region of interest, thereby providing the most reliable temporal reference for the earliest otolith growth of adult tuna.

The selected 0+ aged otoliths used to establish the reference curve (60 from each species) will be sampled manually by isolating the first few months of growth using well-established sectioning and grinding techniques. Andrews et al. (2020) utilized a multi-step approach to core isolation that led to extraction of several months of material in a verifiable manner (Figure 2). This otolith material will be processed using a state-of-the-art system that uses gas-AMS, as opposed to graphite-AMS (sample loss during this process), which is a major step forward in terms of increased efficiency and precision for sample masses that are on the order of 10 times smaller than required for other methods (see Andrews et al. 2019) — this approach avoids the potential problems associated with the inclusion of more recently formed material (see Ishihara et al. (2017) for potential problems with large core extraction masses). In addition, the sample handling time is reduced by eliminating the graphitization step and thereby decreases costs without loss of precision.

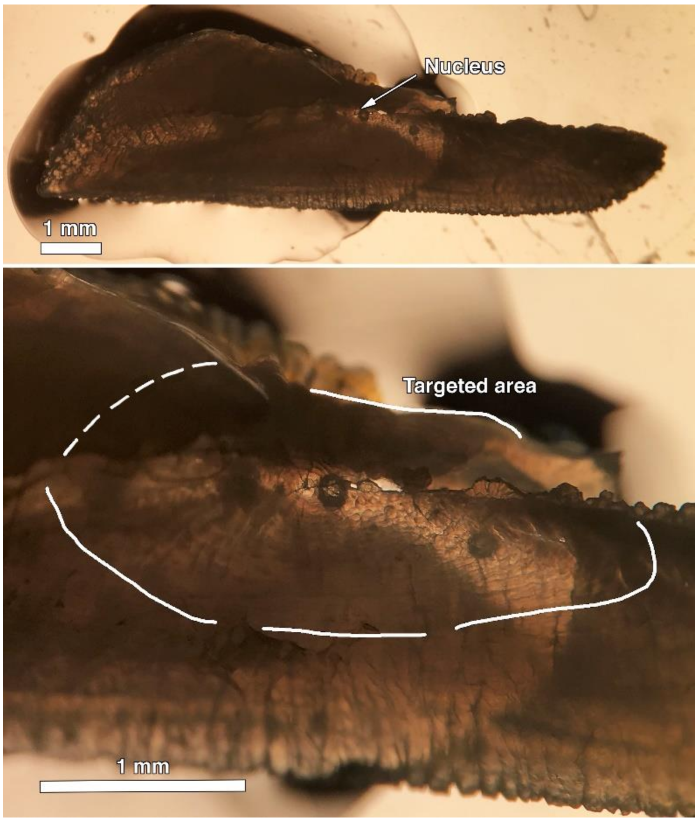

Figure 2. Whole yellowfin tuna otolith with close-up showing how the otolith core will be targeted, as was demonstrated by Andrews et al. (2020). Target area was estimated to be several months of growth based on daily increment observations.

The series of adults used to test the validity of age estimates (40 YFT and 100 BET) will be cored in the same manner as stated above and the measured ^{14}C levels compared to the juvenile otolith reference curve. The variability of the decline reference record will be reduced by comparison of the slopes and intercepts of the respective decline regressions and the concordance of adult ^{14}C data within the 95% prediction intervals of the reference (Figure 3; Andrews et al. 2020).

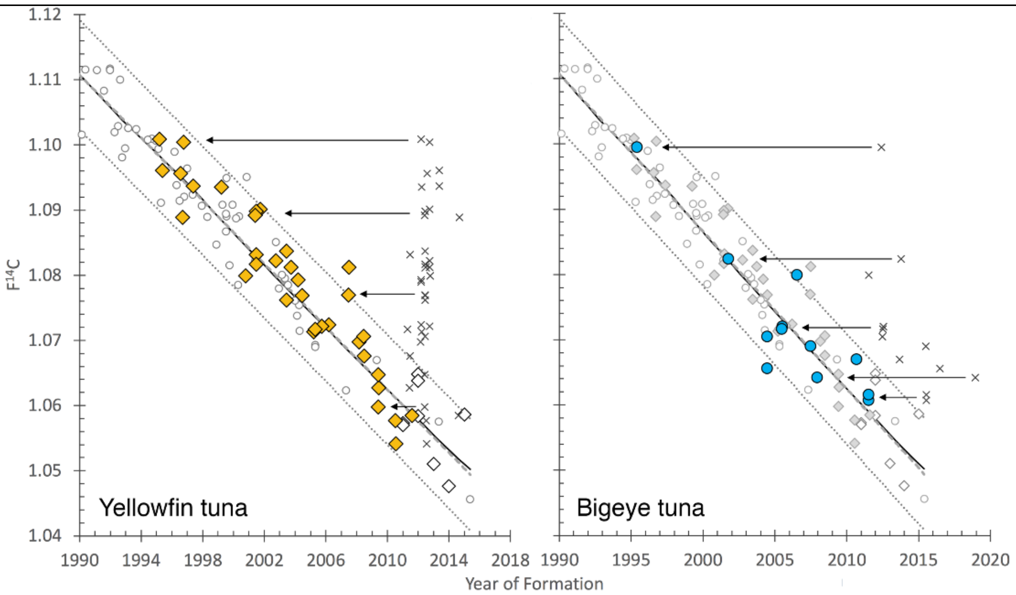

Figure 3. Plot of adult YFT and BET otolith core measurements as show at collection (X's) and projected back to estimated birth years from age estimates made using growth zone counting. These data are compared with a consistent bomb-produced ^{14}C decline reference consisting of coral and otoliths, of which juvenile YFT are included and aligned (Andrews et al. 2020).

In addition to the analysis of otolith material for ^{14}C levels via gas-AMS, it is proposed that a pioneering technology be used to investigate the uptake of ^{14}C within adult BET otoliths through ontogeny. The recent development of gas-AMS by members of the Ion Beam Physics Lab (ETH Zürich; Mini Carbon Dating System (MICADAS) by Ionplus (https://www.ionplus.ch/micadas)) has led to a laser ablation (LA) adaptation for continuous measurement of ^{14}C from a carbonate sample scan (shells, speleothems, deep-sea coral; Welte et al. 2016). This innovative method has been extended to include measurement of a complete bomb-produced ^{14}C signal within the otoliths of individual red snapper, providing evidence of a 60-year lifespan (Andrews et al. 2019). Of interest for this approach are the potential changes in the uptake of ^{14}C to the otolith of vertically migrating BET. The questions are: 1) does the uptake of ^{14}C to the otoliths of BET change though ontogeny as the species increasingly occupies cooler waters (expected to be ^{14}C-depleted), and 2) can the much smaller otoliths be used in LA-AMS to reveal these changes through time and provide a relation to age? As part of this proposal, whole otoliths of three older adult BET will be mounted in an exploratory manner to be scanned for ^{14}C with LA-AMS across the growth axes.

This work will provide unprecedented results for BET and YFT in the WCPO with ^{14}C baselines that can be utilized in numerous future studies of the pelagic environment. The resulting manuscripts and peer-reviewed publications will provide new information on the use of BET and YFT otoliths in estimating growth parameters and potentially monitoring changes in stock age-structure through time. The bomb ^{14}C reference can lead to use of this technique with other pelagic fishes, such as other tunas (i.e., skipjack, albacore), billfishes (e.g., blue marlin; Andrews et al. 2018), and a potentially sharks (e.g., oceanic whitetip; Passerotti et al. *In review*). In addition, the shared technology will open avenues to other working groups to pursue use of methods that provide greater precision on smaller sample masses, coupled with the revelation of LA-AMS technology, in other life history studies of the marine environment.

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Timeframe	12 months.
Budget	A total budget request from WCPFC is 97,980 USD Salary (AH Andrews): 65,000 USD Salary (CSIRO): 7,500 USD Travel (2 weeks @ ETH Zürich, AH Andrews): 8,580 USD AMS analyses (ETH Zürich): 42,900 USD Laser ablation-AMS (ETH Zürich): 3,000 USD Supplies/Equipment: 6,000 USD Unspent from Project 98: (-35,000 USD*) *Note that funding for Project 98 (35,000 USD) was not spent as the workshop was held online. The funds can contribute to the project costs. Note that this project budget covers the work by Dr. Allen Andrews and CSIRO. It does not cover costs of other project partners.

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