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**Progress report on bomb radiocarbon age validation for
bigeye and yellowfin tunas in the WCPO (Project 105)**

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A. Andrews¹, K. Okamoto², K. Satoh^{2,3}, F. Roupsard⁴, J. Farley⁵

¹ University of Hawaii, Manoa, Hawaii

² National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Resources Institute, Shizuoka-shi, Japan

³ National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Resources Institute, Yokohamashi, Japan

⁴ Pacific Community, Noumea, New Caledonia

⁵ CSIRO Oceans and Atmosphere, Hobart, Tasmania, Australia

Progress report on bomb radiocarbon age validation for bigeye and yellowfin tunas in the WCPO (Project 105)

Allen Andrews¹, Kei Okamoto², Keisuke Satoh^{2,3}, Francois Roupsard⁴, Jessica Farley⁵

1. Executive summary

This paper describes the progress made for Project 105 aimed at using bomb radiocarbon (¹⁴C) dating to test the validity of age estimates from purported annual growth zones in otolith sections of yellowfin (YFT) and bigeye (BET) tuna of the western central Pacific Ocean. A total of 123 otoliths were selected from archived young-of-the-year (yoy) samples to establish a bomb ¹⁴C reference record that will cover potential birth years of recently collected adult tuna, given ages into teenage years are accurate. Aged adult YFT and BET that will be analyzed for ¹⁴C from extracted otolith cores (earliest growth) were also selected from the tissue bank to cover the full estimated lifespan. Fish deemed most reliable with high age reading confidence, coupled with otolith mass as a discriminating factor, led to selection of a series of 80 YFT specimens (ages 2–14 years) and 60 BET (ages 2–13 years) that will provide the best results for each species.

2. Introduction

As demonstrated in the recent assessments of WCPO BET (McKechnie et al., 2017; Vincent et al., 2018; Ducharme-Barth et al. 2020), the specification of growth in integrated stock assessment models, such as MULTIFAN-CL, can have profound effects on stock status indicators. Hence, it is essential that such assessments use the best growth data and/or growth model estimates available. 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 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 age. Direct age validation of the otolith age reading was made through an analysis of several strontium chloride (SrCl₂) marked tuna otoliths that were tagged and recaptured. This validation is relatively limited, particularly for YFT, and a recent workshop held at IATTC on BET and YFT growth made the following conclusion (Farley et al., 2019): “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.”

¹ University of Hawaii, Manoa, Hawaii

² National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Resources Institute, Shizuoka-shi, Japan

³ National Research and Development Agency, Japan Fisheries Research and Education Agency, Fisheries Resources Institute, Yokohama-shi, Japan

⁴ Pacific Community, Noumea, New Caledonia

⁵ CSIRO Oceans and Atmosphere, Hobart, Tasmania, Australia

Recently, annual age reading protocols for YFT and BET in the western North Atlantic - 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 ^{14}C levels in a ^{14}C reference series, often a validated coral core chronology, for the region of interest to determine if the calculated birth year from otolith growth zone counts is consistent with the ^{14}C reference. At the SPC pre-assessment workshop in 2020, the bomb radiocarbon method was presented using BET 0+ aged fish (i.e. yoy) from the WCPO to investigate the distribution of ^{14}C in otoliths through time (Hamer and Pilling, 2020). The results were promising — there was a decline in the measured ^{14}C values that was similar to regional coral ^{14}C reference records. Figure 1 shows the yoy BET ^{14}C values from that work compared to all known regional coral and known-age otolith ^{14}C data. Hence, an expansion of this line of research was proposed and funded by the WCPFC to make a full application of bomb radiocarbon dating to BET and YFT of the WCPO (Farley et al., 2020b).

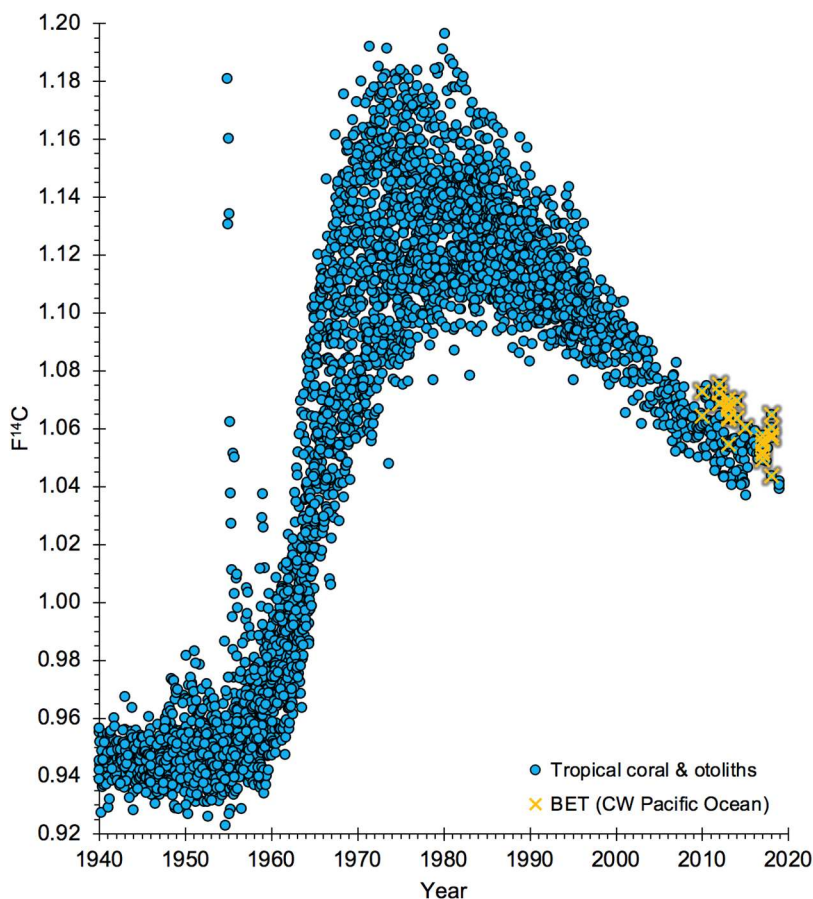


Figure 1. Plot of all known regional coral and known-age otolith ^{14}C data from the tropical and subtropical Pacific Ocean that can be used as reference material with the results of ^{14}C measurements from 0+ aged BET (yoy). These results were previously performed by the National Research Institute of Far Seas Fisheries and highlight the position of tuna otolith ^{14}C reference material as residing toward the more elevated side of the broad cross section of Pacific Ocean ^{14}C reference records.

3. Objectives

The purpose of the project is to test the validity of age estimates for bigeye and yellowfin tuna from the WCPO using bomb ^{14}C dating.

The project will:

- 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 using otoliths of juvenile YFT and BET tuna collected through time from the WCPO.
- Investigate the uptake of ^{14}C within adult BET otoliths through ontogeny using laser ablation -accelerator mass spectrometry (LA-AMS) technology, in addition to the analysis of otolith material for ^{14}C levels via gas-AMS.

4. Progress against objectives

Reference curve for bomb-produced ^{14}C

Prior to selection of yoy tuna otoliths for use as a ^{14}C reference, an analysis of all existing coral and known-age otolith records from the subtropical and tropical Pacific Ocean were assembled (Figure 1). This collective record built upon an analysis of temporal constraints for mixed layer ^{14}C levels across this broad region from which a 3.71 m LJFL (565 kg) blue marlin (*Makaira nigricans*) was aged to 20 years (Andrews et al., 2018). Since the time of the blue marlin research, additional ^{14}C records have become available from both coral and otoliths of juvenile fish and were added to this study. Included in this series is a recently accepted coral ^{14}C reference from American Samoa that extends to 2012 and provides an analysis of DIC ^{14}C in the context of South Pacific Gyre oceanography (Andrews et al., *In Press*). The collective ^{14}C reference record covers a geographical area that ranges northward to the Hawaiian Islands and southward to Easter Island and from the southern Great Barrier Reef to numerous records across the far western central Pacific. While there are constraints to potential years of formation for otoliths across this broad section of the Pacific Ocean, there is room for regional variability in the more time-specific, post-peak ^{14}C decline period — it is the use of yoy tuna otoliths that will provide a refinement of the temporal specificity, which was not available for the 20-year estimate made for the 3.71 m blue marlin.

The priority of this study was careful selection of the yoy otolith series to be used as an expansion of the existing BET reference series (collection years 2010–2018; Figure 1). WCPFC, CSIRO and SPC Tuna Tissue Bank (TTB) (see SPC-OFP 2021 [SC17-RP-35b-01] for TTB details) archives were fully canvassed for both BET and YFT juveniles that were either previously aged with daily increment counts or were assumed to be small enough in terms of body size (<500 mm FL) to be less than 1 year old — emphasis was placed on using the smallest fish first (near 300 mm FL). The selection was expanded to include larger fish as necessitated by the desire to establish a comprehensive series of years with ^{14}C measurements. A total of 123 otoliths were selected within a collection period of 1990 to 2019 to cover potential birth years of recently collected adult tuna (given ages into teenage years are accurate) and are represented by 52 BET, 61 YFT, and 10 skipjack (*Katsuwonus*

pelamis) yoy otoliths that are in good condition or have an otolith core that is intact. Otoliths for YFT were more prevalent and therefore outnumber the selected BET otoliths, and 10 SKJ otoliths were selected to fill in some regional years that were not well represented, but also to add ^{14}C observations for a third species distributed across the same geographic region.

The regional coverage of the available yoy specimens was also a factor in considering its inclusion as a reference measurement for otolith ^{14}C . The existing BET reference series (Figure 1) covered a geographical range of 5°N – 8°S and 144°E – 164°E . The latitudinal and longitudinal range was expanded within the selected otoliths for this study to cover a broader range of natal origins across the Central Western Pacific and range from 4°N to 40°S and 140°E to 127°W . Most yoy were from a narrower geographical range (4°N – 9°S , 140°E – 180°) with a few from more distant locations to the east and south to assess potential variability in ^{14}C uptake by the otolith between locations.

The yoy tuna otoliths have been assembled after frequent communications among project partners about the availability and condition of selected specimens. In many cases, either the preferred otolith was not available or in a condition that may compromise or prevent accurate core extraction. These otoliths have been delivered and verified, checked for condition, and photographed to create an accurate record of the otoliths used in this study. At present, supplies that are necessary for mounting and extracting the otolith material are taking longer than is usual to be delivered due to the Covid-19 epidemic.

Testing the validity of adult YFT and BET age and longevity estimates

The second priority in this project is to select and analyze otoliths of aged adult BET and YFT for ^{14}C across the full estimated lifespan. The TTB archives were again fully canvassed for available specimens from each species that were aged and deemed most reliable with high age reading confidence. Within these selection criteria, a combination of collection year and lifespan coverage were used to focus on a series of otoliths that would effectively trace the post-peak ^{14}C decline back in time such that the slopes of the yoy ^{14}C reference series can be compared with the ^{14}C decline determined from adult otolith cores. The goal is to provide either an alignment or misalignment of the sample series with the post-peak ^{14}C decline to better assess the age reading protocol, an approach similar to what was successfully performed for YFT in the Gulf of Mexico (Andrews et al., 2020).

Otoliths were selected based on the above criteria coupled with otolith mass as a discriminating factor (Figure 2). These processes resulted in the final selection of 80 YFT specimens (ages 2–14 years) and 60 BET (ages 2–13 years) that will cover what appear to be well developed growth functions (Figure 3) and will provide the best results for each species. The original focus was on BET, but it has been determined that both species can be assessed well with the numbers selected. While the plan is to continue to look for more older BET based on otolith mass (see analysis in Andrews et al. (2020)), we assume it is unlikely that we will find additional specimens and will proceed with what we know is

available for YFT and BET. If other BET otoliths that may provide better coverage of the estimated lifespan are located, they can be easily shifted into place by removing less informative specimens at a later date. Hence, we propose that more fish are selected from the YFT archives because a more thorough selection of aged fish is available. It is important to consider that despite having fewer fish for BET in this study, the number of ^{14}C measurements is outstanding considering there were 34 YFT (2–18 years) in the successful study of YFT from the Gulf of Mexico (Andrews et al., 2020).

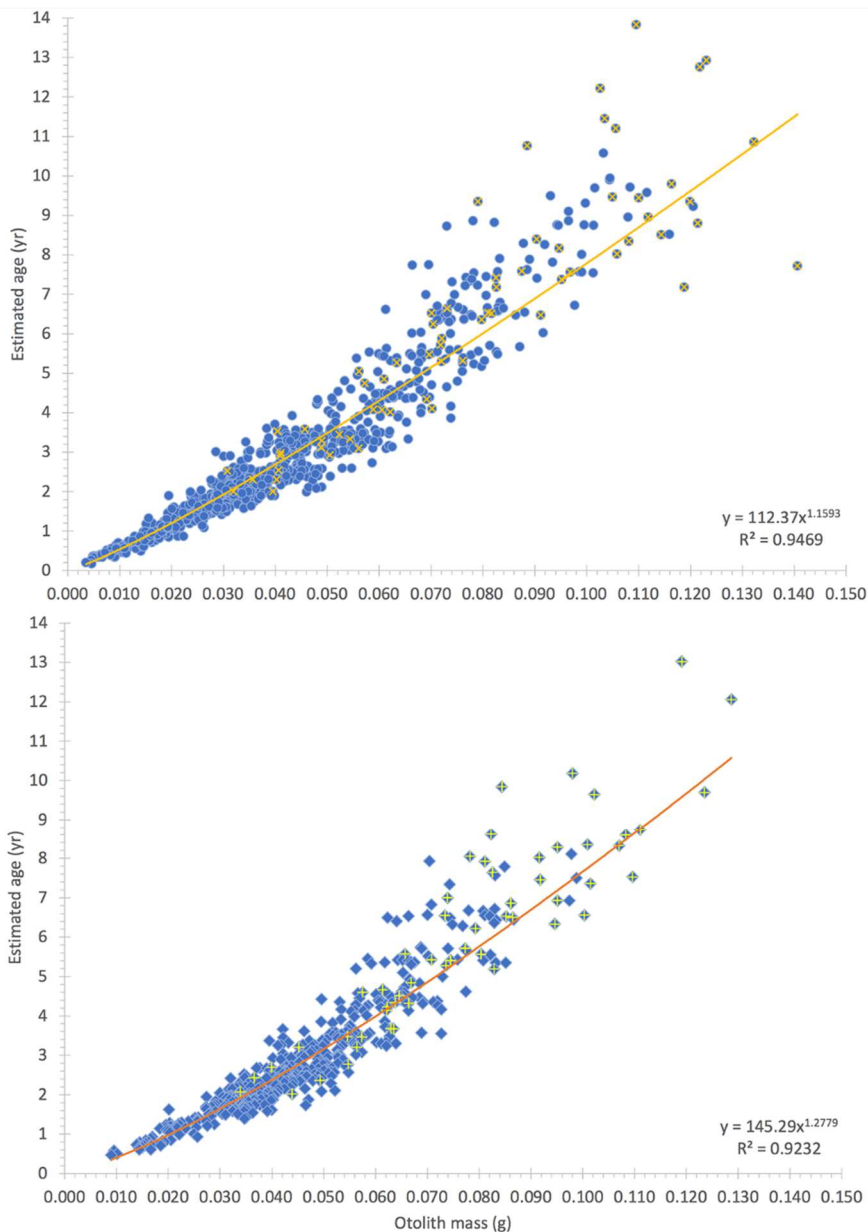


Figure 2. Plot of YFT and BET otolith data where both estimated age and otolith mass were available. Selected within these data sets are the otoliths for ^{14}C analyses with an x (YFT, top) or + (BET, bottom) for ages 2–14 years. Otoliths were chosen in an ad hoc manner on the basis of representing an otolith mass range for the age groups, except the oldest fish (>10 yrs for YFT and >9 yrs for BET), which were all selected.

The selected otoliths of aged adult YFT and BET use all of the oldest fish available (within the selection criteria stated above) because fewer fish were available and maximum age is an important part of testing the validity of the age reading protocol. For YFT, there were 15 fish aged to >10 years and 8 fish age to >9 years for BET. Younger adults were selected in an ad hoc manner with the aim of representing an otolith mass range within arbitrary age groups. The additional YFT adults are represented by 30 fish aged 7-9 years, 20 fish aged 4-6 years, and 15 fish aged 2-3 years for a total of 80 fish. The additional BET adults are represented by 22 fish aged 7-8 years, 15 fish aged 5-6 years, and 15 fish aged 2-4 years for a total of 60 fish. Sex was also considered within each age group with females selected first when available in the largest size classes (males tend to be larger than females). Close to an equal split was chosen for the younger age groups for both YFT and BET. To assist with visualizing how length-at-age is represented by the otoliths selected for bomb ^{14}C dating, a plot was made using the measured fish length and estimated age for all available fish with selected samples highlighted (Figure 3).

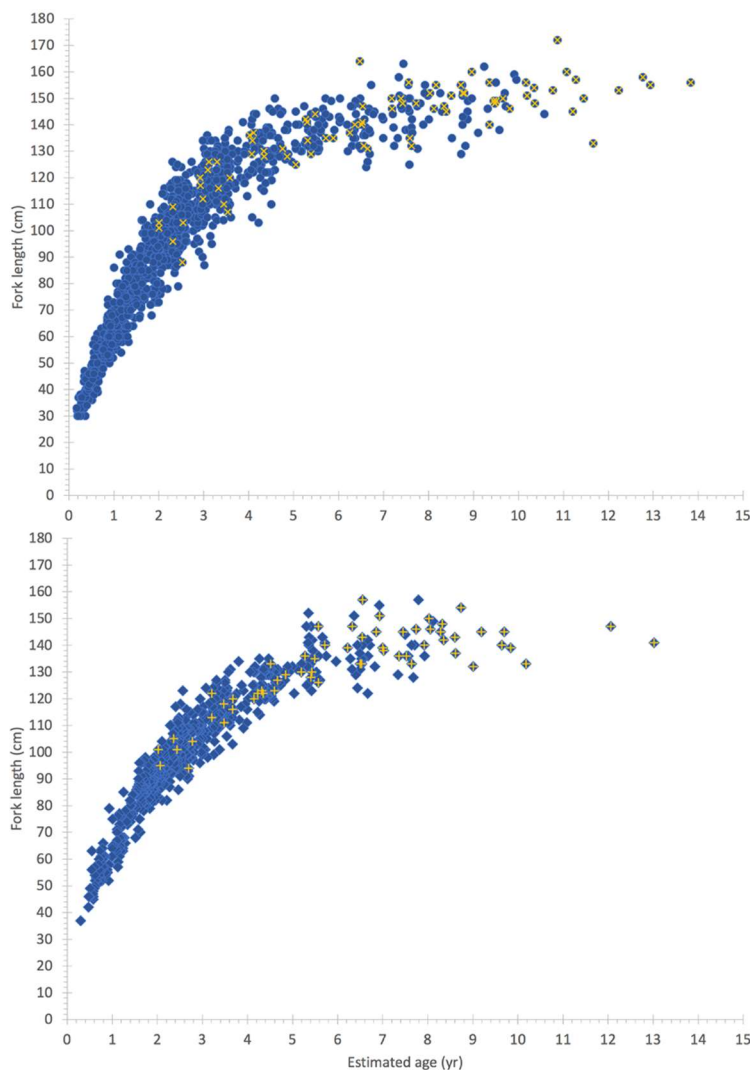


Figure 3. Plot of YFT and BET length-at-age from measured fish that were aged using the annual age reading protocol that is being tested with bomb ^{14}C dating (blue diamonds). The selected otoliths for ^{14}C analyses are shown with a gold x (YFT, top) or gold + (BET, bottom) for ages 2–14 years and covering the range of available fish lengths within the arbitrary age groups.

5. Work plan

The current plan for the yoy YFT and BET selected for analysis is to use a multi-step approach to isolate the first few months of growth (around the core) in a verifiable manner as described by Andrews et al. (2020). 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 (Andrews et al., 2019) — this approach avoids the potential problems associated with the inclusion of more recently formed material.

Selected adult otoliths aged 2–14 years will be gathered and inspected for condition. Once verified as suitable for bomb ^{14}C dating they will be sent to the lab for processing and core extraction. Among these samples, a few will be selected and prepared for an experimental application of laser ablation AMS at ETH Zürich 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 through 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? The plan is for Dr. Andrews to visit and work in the Ion Beam Physics lab with Dr. Caroline Welte to pursue successful measurements across the small otoliths. Travel restrictions appear to be waning and it is hoped that this can occur later in 2021 or the first part of 2022.

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