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**Progress report on bomb radiocarbon age validation for
yellowfin and bigeye tunas in the WCPO (Project 105) - 2022**

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Progress report on bomb radiocarbon age validation for yellowfin and bigeye tunas in the WCPO (Project 105) - 2022

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1. Executive summary

This paper describes the progress made for Project 105 aimed at using bomb radiocarbon (¹⁴C) dating to test estimates of age from purported annual growth zones in otolith sections of yellowfin (YFT) and bigeye (BET) tuna of the western central Pacific Ocean. A total of 134 otoliths from archived young-of-the-year (yoy) samples were selected and submitted for accelerator mass spectrometry (AMS) analysis. Of these samples, a total of 90 have been analysed to date to establish a bomb ¹⁴C reference record that covers potential birth years of recently collected adult tuna, given ages into teenage years are correct. The yoy tuna ¹⁴C time series has shown strong concordance thus far with existing coral-otolith ¹⁴C reference records from the tropical and subtropical Pacific Ocean. A series of 142 otoliths from older YFT aged 1–14 years and BET aged 1–13 years were sampled for the earliest growth (core extraction) and submitted for ¹⁴C analysis with results obtained for 72/77 YFT and 61/65 BET to date, of which half were recently completed (mid-July) on the AMS. Alignment of estimated birth years for older fish with the yoy ¹⁴C reference is good for YFT and BET and further analyses are necessary to finalize the work. The delay to finishing this project is due to longer than anticipated time necessary to select and locate useful otolith specimens for older fish from the archives, an assortment of Covid-19 related problems, and competition for AMS use at ETH Zürich. This report covers the details of what has been completed and what remains.

2. Introduction

As demonstrated in the recent assessments of western and central Pacific Ocean (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 analyse 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

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chloride (SrCl_2) 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.”

Annual age reading protocols that have generated ages, typically greater than previous studies, for YFT and BET in the western North Atlantic - Gulf of Mexico were validated using bomb ^{14}C dating (Andrews et al., 2020). An innovative approach to the method used the post-peak bomb ^{14}C decline period (~1980–2000) to successfully corroborate YFT aged 2 to 18 years and BET aged 3 to 17 years with validated lifespan to 16 years for each species. This new methodology is well-suited to shorter lived species and was recently applied successfully to Pacific bluefin tuna (PBT; Ishihara et al., 2017). The approach compares otolith ^{14}C levels in the core (earliest growth) with a ^{14}C reference time-series for the region of interest, often a date-validated coral core chronology (e.g., Andrews et al., 2012, 2013), to determine if the calculated birth year from otolith growth zone counts is consistent with the ^{14}C reference. In general, an age reading protocol is considered valid if the collective birth years from across all age classes provides no bias relative to the ^{14}C reference chronology (i.e., an offset of the measured otolith ^{14}C values in time). While measured ^{14}C levels from an individual fish cannot provide a specific validated age due to uncertainty within the post-peak reference, it is the combined and replicated age reading across all estimated age classes and their overall alignment that can be used to corroborate the protocol used to estimate age.

In the Project 105 progress report of 2021 (Andrews et al., 2021a), following the bomb radiocarbon age validation workshop in 2020 (Farley et al., 2020b), the bomb radiocarbon method was presented as a preliminary study using BET 0+ aged fish (i.e., young-of-the-year; yoy) from the WCPO to investigate ^{14}C in otoliths through time. The results were promising because the decline in measured ^{14}C values was similar to regional ^{14}C reference records, although elevated overall relative to the central tendency of the coral-otolith ^{14}C reference decline. These ‘original’ yoy BET ^{14}C values are illustrated in Figure 1 in a comparison with all known regional coral and known-age otolith ^{14}C data (Andrews et al., 2018, 2021b). Also shown are the yoy values after being ‘adjusted’ due to a documented ambiguity in reporting of measured ^{14}C values (Reimer et al., 2004) — the back calculated $F^{14}\text{C}$ values were initially made assuming the reported $\Delta^{14}\text{C}$ values were date-corrected. Reconsideration of the raw data led to the proper determination of $F^{14}\text{C}$ values that are now correctly comparable to the ^{14}C reference values (see original and adjusted data in Figure 1). In addition, it is important to note that $F^{14}\text{C}$ is preferred as a temporal reference in date calibrations because it eliminates the need for time-correction to the date of formation of the measured ratio (this introduces circularity in the determination of validated ages) and avoids the common problem in the literature of ambiguous $\Delta^{14}\text{C}$ values that may or may not be date corrected (Reimer et al., 2004). This adjustment led to an overall downward shift of the ^{14}C values toward a strong concordance of the preliminary BET yoy measurements with

the coral-otolith ^{14}C reference chronology (Figure 1). This adjustment is investigated further in a comparison with the yoy BET and YFT findings for the current study, and ultimately a combination of data sets for the BET ^{14}C reference.

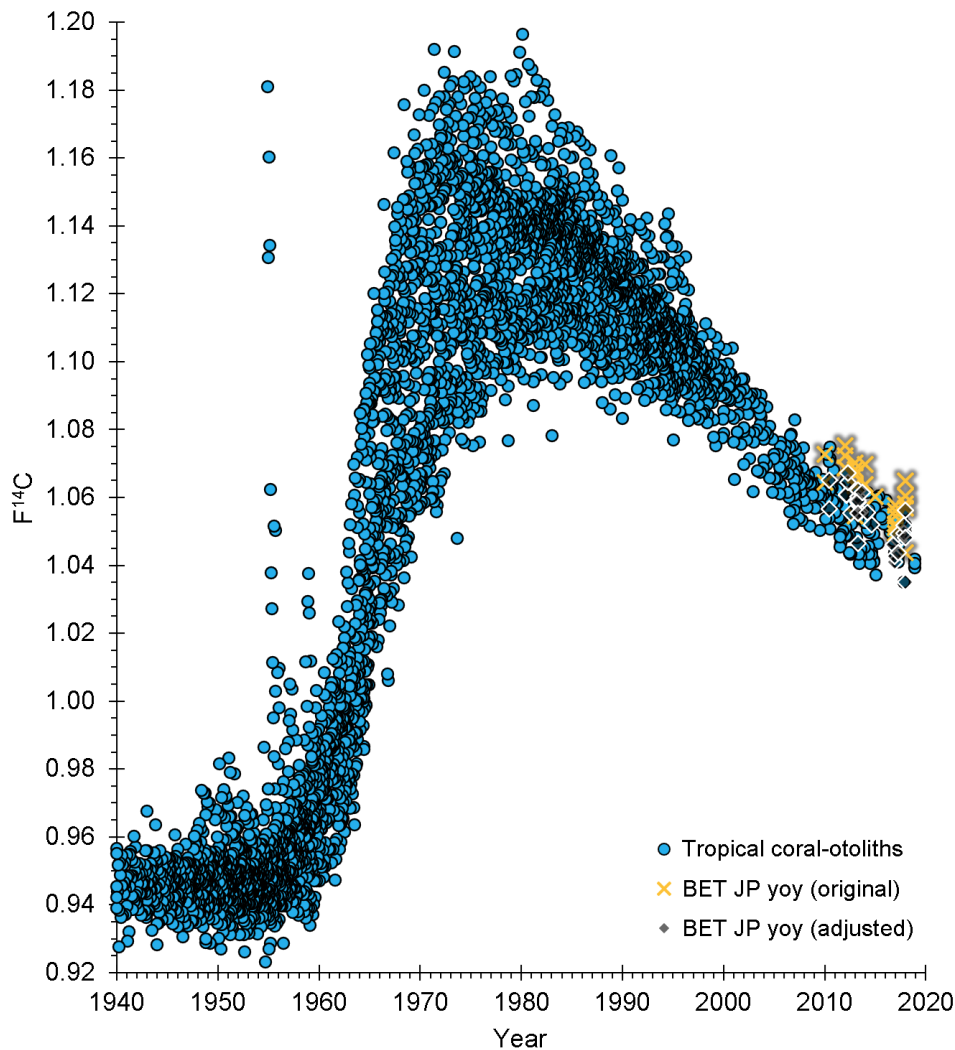


Figure 1. Plot of all known regional coral and known-age otolith ^{14}C data from the tropical and subtropical Pacific Ocean that can be used as reference material (Andrews et al., 2018, 2021b) with the preliminary results of ^{14}C measurements from 0+ aged BET (yoy). The original and adjusted $F^{14}\text{C}$ values reveal a well-documented problem in the reporting of $\Delta^{14}\text{C}$ values (Reimer et al., 2004). Note the strong concordance of the adjusted yoy BET values with the coral-otolith chronology. These results were part of a previous study performed by the National Research Institute of Far Seas Fisheries (Currently Fisheries Resources Institute), Japan (JP).

3. Objectives

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

The project will:

- Establish a reference time series for bomb-produced ^{14}C that will provide a baseline using otoliths of juvenile YFT and BET tuna collected through time from the WCPO.

- Test the validity of adult YFT and BET age and longevity estimates, as well as the age reading protocol, with the new regional ^{14}C reference chronology.
- Investigate the uptake of ^{14}C within individual YFT and BET otoliths through ontogeny using the rostrum tip of large adults in comparison with the otolith core (this is a substitution for LA-AMS due to instrument limitations).

4. Progress toward 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 cores 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., 2021b). 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 locations 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 provides confirmation of the temporal specificity of ^{14}C levels on the decline for tuna, which was not an option for the 20-year estimate made for the 3.71 m blue marlin.

The priority of this part of the 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 Pacific Marine Specimen Bank (PMSB; see SPC-OFP 2021 [SC17-RP-35b-01] for PMSB 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 (<50 cm FL) to be less than 1 year old — emphasis was placed on using the smallest fish first (near 30 cm 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 134 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 may be represented by 62 YFT, 54 BET, and 18 skipjack (SKJ; *Katsuwonus pelamis*) yoy otoliths that were in good condition or have an otolith core that was intact (extra specimens were selected to cover possible sample loss). Otoliths for YFT were more prevalent and therefore outnumber the selected BET otoliths, and the additional 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. A total of 90 yoy tuna samples have been processed to date.

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 21°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 (Figure 2).

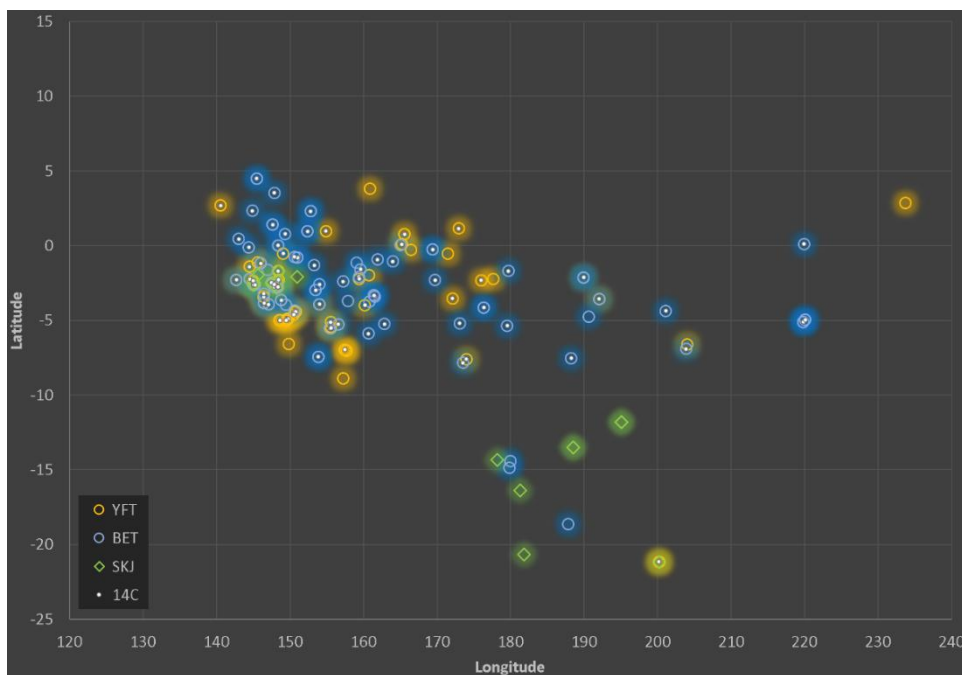


Figure 2. Distribution of yoy YFT, BET, and SKJ selected for the tuna ^{14}C reference chronology. Those with measured ^{14}C values are highlighted with a white dot. Latitude ranges from 15°N to 25°S (-25) and longitude ranges from 120°E to 120°W (240). Colour shading intensity is an indication of location sampling replicates.

The yoy tuna otoliths were 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 was in a condition that may compromise or prevent accurate core extraction. The finalized otolith selections were delivered and verified, checked for condition, and photographed to create an accurate record of the otoliths used in this study. Supplies that were necessary for mounting and extracting the otolith material took months longer than is usual to be delivered due to the Covid-19 pandemic, but all specimens were eventually prepared with more than required numbers to cover potential losses.

Extraction of the earliest growth, similar to what was later performed for the older adults, was the target for measurement of ^{14}C as tuna reference material. Each otolith was prepared as described by Andrews et al. (2020). Microscopic examination of extracted

otolith material, coupled with containment using flexible mounting media, led to confidence that the earliest growth (likely the first few months of life based on daily increments observed in many of the whole otoliths) was isolated from the otolith specimen. Measured ^{14}C values from sample masses of 0.2–0.8 mg CaCO_3 were successful for almost all submitted samples, but additional samples remain in the queue due to unavoidable delays to AMS access.

The ^{14}C time series from YFT and BET yoy otoliths revealed a strong concordance with the coral-otolith ^{14}C chronology (Figure 3). Inclusion of the previous BET data set (adjusted ^{14}C values) strengthened the post-peak decline relationship. A preliminary analysis of each yoy data set with a simple linear regression led to a unison decline rate of 2.1–2.2 per mille per year and a spatial-temporal distribution that was within the constraints of the regional coral-otolith reference chronology. Further analyses will be performed once all yoy data are available.

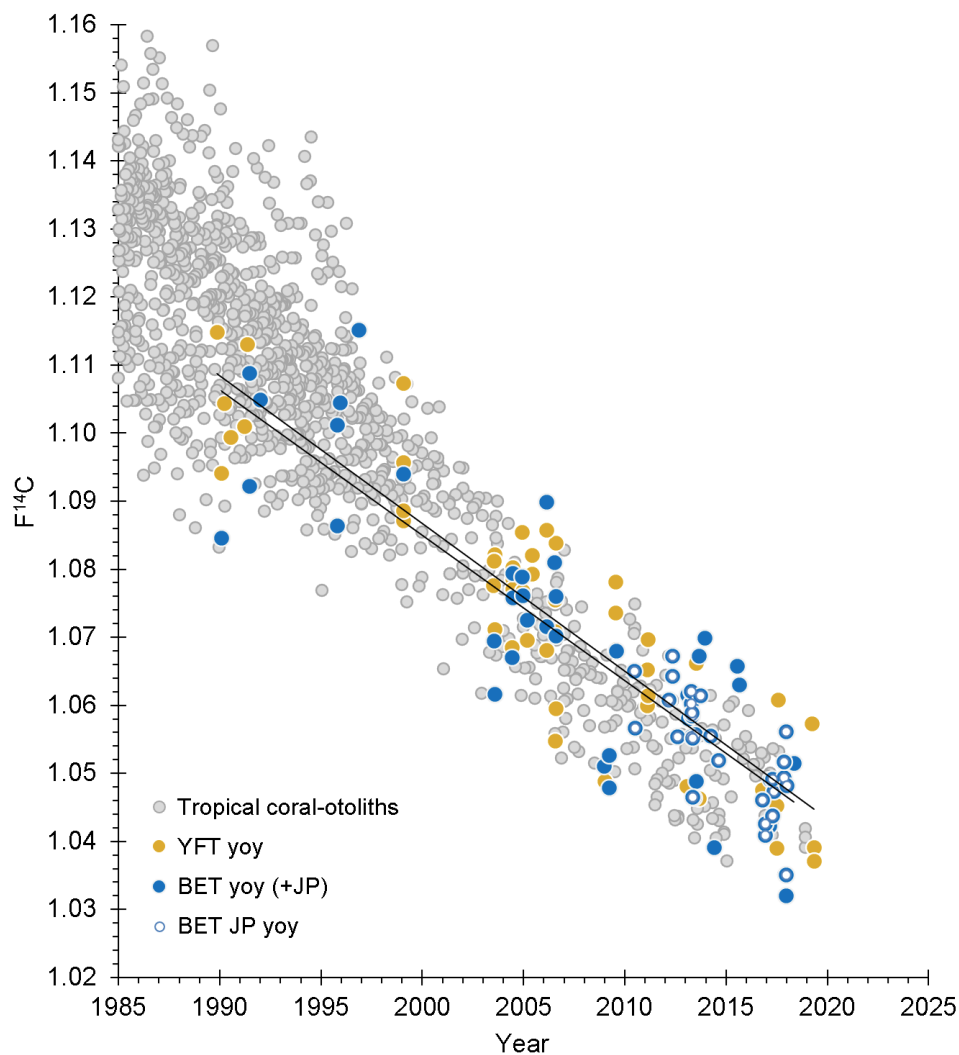


Figure 3. Plot of all ^{14}C measurements to date for yoy YFT and BET across nearly 30 years of collection dates showing a remarkable correlation with the regional coral-otolith ^{14}C reference chronology. Included are BET yoy from a preliminary study (BET JP; see Figure 1) that strengthen the post-peak decline relationship (symbol differentiated to show adjusted alignment). Each species demonstrated a similar post-peak relation

that is useful in testing the validity of age estimates for adult tuna from otolith core ^{14}C measurements. The preliminary, nearly coincident, regressions are shown for yoy YFT and BET (JP samples included). These relationships will be analysed further relative to each other and the coral-otolith reference when all yoy samples have been processed.

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

Selected and analysed otoliths of aged adult YFT and BET for ^{14}C across the full estimated lifespan is nearly complete, with good results for YFT and BET. The delay to finishing this project is due to longer than anticipated time necessary to select and locate useful otolith specimens for older fish from the archives, an assortment of Covid-19 related problems, and competition for AMS use at ETH Zürich.

The PMSB 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 determine whether the decline in the ^{14}C time series for older YFT and BET aligns with the post-peak ^{14}C decline to 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 (Andrews et al. 2021a). The result was a selection of more than 70 YFT and more than 60 BET adults to cover what appears to be well developed growth functions and would likely provide the best results for each species (Andrews et al. 2021a). The original focus of the proposal was on BET, but fewer were available overall and it was determined that both species could be well assessed with the numbers selected. As a result, 142 otoliths from older YFT aged 1–14 years and BET aged 1–13 years were cored (earliest growth extracted) and analysed for ^{14}C , with results obtained for 72/77 YFT and 61/65 BET to date, of which half were recently completed (mid-July) on the AMS. The geographical distribution covered by the selected samples is illustrated along with the overall yoy tuna reference samples from this study (Figure 4).

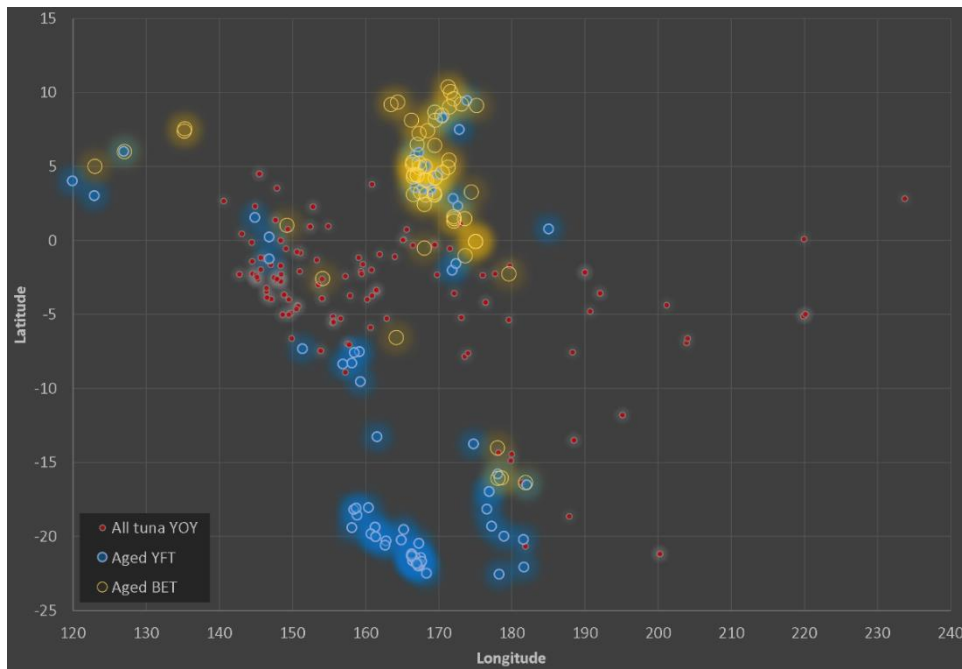


Figure 4. Distribution of collection locations for the aged YFT and BET selected for age validation along with the yoy tuna ^{14}C reference chronology locations (Figure 2). Latitude ranges from 15°N to 25°S (-25) and longitude ranges from 120°E to 120°W (240). Colour shading intensity is an indication of location sampling replicates.

The overall alignment of the older adult YFT with the coral-otolith reference chronology, and most importantly the yoy YFT time-series, is compelling for corroboration of the estimated ages and the age reading scenario to 14 years (Figure 5). Of the 72 measured ^{14}C values to date, only four clearly reside outside the expected birth years established by the ^{14}C references (Figure 5). One value was lower than can be accounted for by the variability in otolith uptake and may be attributed to either over-estimated age by at least 3 years (aged to 11.2 years but may have been less than 8 years old) or an inadvertent inclusion of newer (more recently formed) otolith material during the core extraction process. When considering the overall agreement of the other older aged fish and otolith mass (a reasonable proxy for age), it seems likely there was a problem with the sample composition as opposed to age estimation. Three other values were similarly offset but to greater ^{14}C values. The two most elevated would need to be 3 to 5 years older to begin to align with the reference chronology (aged 3.3 years but would need to be 6–7 years old, and aged 9.7 years but would need to be 15 years old, respectively). The sample with the greatest offset would need to be 6 years older than an estimated age of ~1.5 years, which is unlikely for a 79 cm FL fish. Hence, it is probable that there was a problem with the sample or ^{14}C measurement that led to these offset values, as opposed to a problem with underestimated age. Overall, the coincidence of the data sets for 68/72 measurements and similar slopes ($m = 2.0$ cf. 2.2 per mille ^{14}C per year) for this stage of the study is an indication that lifespan estimates of 14 years for YFT are supported and that the age reading protocol shows no bias toward younger or older ages.

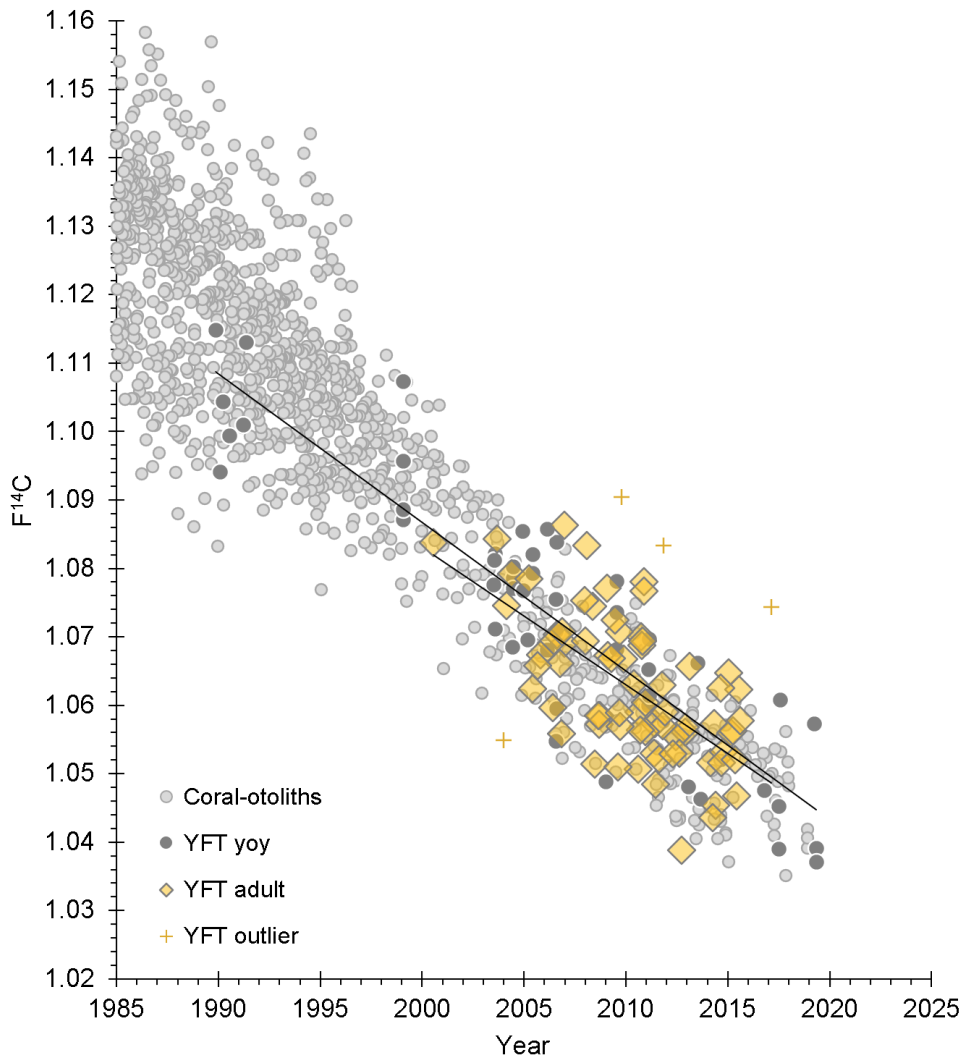


Figure 5. Plot of otolith ^{14}C data for aged YFT with the yoy YFT and regional coral-otolith reference chronologies. The ^{14}C measurements from older YFT otolith core material were in alignment with the coral-otolith reference series and the yoy YFT series, exhibiting a similar decline rate and spatial-temporal concordance. Of the 48 otolith core samples measured thus far, only four were considered outliers. Hence, the series of purported birth years using the new otolith age reading protocols up to 14 years are well supported.

The results for older BET indicate a similar scenario to YFT (Figure 6). The coincidence of the data sets for 59/61 measurements and similar slopes ($m = 2.1$ *cf.* 2.4 per mille ^{14}C per year) for this stage of the study is an indication that lifespan estimates of 13 years for BET are supported and that the age reading protocol likely shows no bias toward younger or older ages. Similar to what was observed for outlier YFT samples, two measurements are lower than can be accounted for by the ^{14}C references. The offset to lower-than-expected values could be from inclusion of younger otolith material or overestimated age. Both were from smaller fish at 66 and 126 cm FL aged 1 and 5 years old, respectively, and it is unlikely that overestimated age is an issue. It is more likely that inadvertent incorporation of more recently formed carbonate led to reduced ^{14}C levels. Overall, the trend supports the newer otolith age interpretations and further analysis with conclusions will follow this progress report.

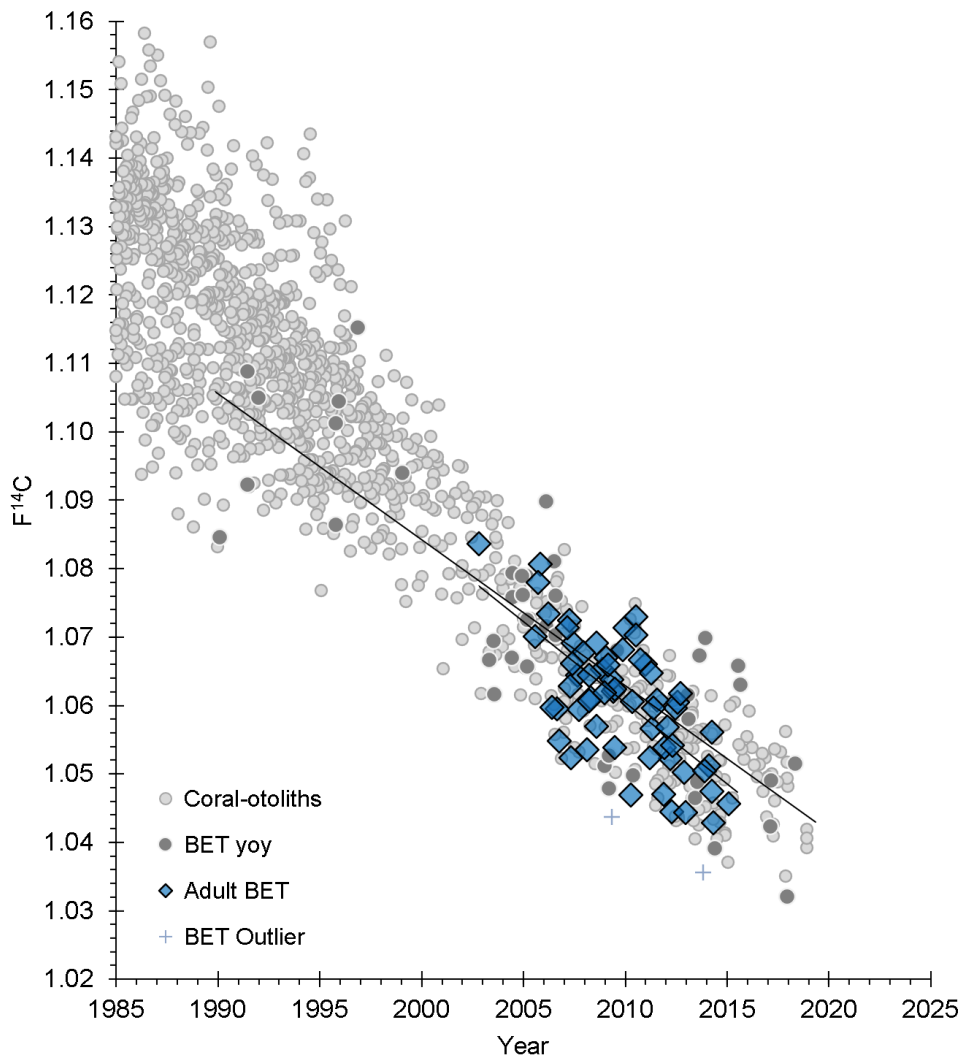


Figure 6. Plot of otolith ^{14}C data for aged BET with the yoy BET and regional coral-otolith reference chronologies. Similar to YFT, two young fish revealed ^{14}C levels that were lower than can be accounted for by age and the reference records. Overall, there is support for the age reading to 13 years.

Substitution for LA-AMS

Laser ablation accelerator mass spectrometry (LA-AMS) technology uses a new apparatus that is being developed by researchers at ETH Zürich to provide continuous measurement of ^{14}C across geologic and biogenic carbonates (Welte et al. 2016). The original plan for the current study was to analyse BET otoliths from several large adults with the most massive otoliths and greatest age estimates to document changes in ^{14}C uptake that may be attributed to residing at greater depths through ontogeny. However, during a recent site visit to the Ion Beam Physics Lab (October 2021), the consensus was that the otoliths of tuna are too small to provide meaningful results from the LA-AMS system. The reasons were due to the following limitations: 1) the otoliths do not exhibit a growth structure that would facilitate a good linear scan with enough depth and width along an age-specific pathway, and 2) preparation of planar sections that contain sufficient material would not be possible based on the required dimensions for laser scanning. While this approach was successful in providing complete bomb ^{14}C signals through ontogeny for the massive otoliths (up to 5 g)

of red snapper (*Lutjanus campechanus*; Andrews et al. 2019), the technology currently does not have the resolution to analyse the small otoliths of tuna (typically less than 0.2 g). Hence, it was concluded that the next best approach was to analyse an additional sample from the rostrum tip from a series of the most massive otoliths to allow a comparison of ^{14}C uptake between earliest and latest otolith growth.

Only a few of the selected otoliths for this analysis have been processed at this time. Several otoliths of both YFT and BET were selected to potentially provide a contrast in ^{14}C uptake between species. The details of this part of the study will be provided upon completion of the remainder of the samples slated for gas-AMS at ETH Zürich.

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