SCIENTIFIC COMMITTEE
TWENTY-SECOND REGULAR SESSION
11-19 August 2026
Apia, Samoa (Hybrid)

PROJECT 129 UPDATE: BUILDING AN AGE-LENGTH DATA STREAM FOR TUNA ASSESSMENTS

WCPFC-SC22-2026-SA-WP12_Rev01
3 August 2026

Submitted by

Corey Wakefield[1], Joseph Widdrington[1], Kyne Krusic-Golub[2], Alexandre Pons[1], Patrick Reis-Santos[3], Bronwyn Gillanders[3], Jessica Farley[4], Simon Nicol[1], Paul Hamer[1]

Rev-01: Added request for additional funding for microscopy and imaging equipment to increase ageing capacity and support the development of rapid ageing methods during the second half of Phase 1 and Phase 2.

[1] Oceanic Fisheries Programme of the Pacific Community (SPC)
[2] Fish Ageing Services (FAS)
[3] School of Biological Sciences, Adelaide University (AU)
[4] The Commonwealth Scientific and Industrial Research Organisation, Australia (CSIRO)

Executive Summary

Project P129 was established to develop the scientific and operational foundations required to build a scalable age–length data stream for tuna stock assessments within the Western and Central Pacific Fisheries Commission (WCPFC). To achieve this, the project aims to establish surrogates for rapid and scalable tuna ageing, including otolith biometric modelling and epigenetic ageing, and progress their implementation into routine sampling programmes. This project addresses a long-recognised deficiency in tropical tuna assessments, namely the limited availability of routine conditional age-at-length (CAAL) data and the associated uncertainty in growth, mortality, recruitment, and stock status estimates.

Substantial progress has been achieved during the first six months of the project, with activities advancing beyond the original Phase 1 objectives. A fundamental requirement of all surrogate ageing approaches is a robust and validated reference age dataset derived from aged otolith sections, which underpins the calibration, training, and evaluation of these methods. Significant developments have been made in the refinement and standardisation of direct ageing methodologies for yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*T. obesus*), including the development of reference collections, independent cross-reading exercises, and quantification of ageing precision. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) analyses have been completed on 164 yellowfin tuna, 153 bigeye tuna, and 74 skipjack tuna (*Katsuwonus pelamis*) otoliths to evaluate the periodicity of opaque growth-zone formation and strengthen confidence in age interpretation. Importantly, the combination of LA-ICP-MS analyses and oblique otolith sectioning is showing considerable promise as a method for ageing skipjack tuna (*Katsuwonus pelamis*), a species for which reliable age interpretation has historically proven challenging.

Building upon these validated age datasets, multivariate age-prediction models are being developed integrating otolith morphometrics, fish length, sex, and spatial variables. Preliminary gradient boosting machine (GBM) ensemble models are demonstrating strong predictive precision for both yellowfin and bigeye tuna. This GBM modelling approach is now being extended to South Pacific albacore (*T. alalunga*), using archived otolith collections for initial model development, followed by expanded otolith sampling and age prediction application across the Pacific ahead of the 2027 stock assessment. This approach demonstrates the potential for rapid, cost-effective, and scalable age estimation to support future age–length sampling programmes.

Project P129 also supports epigenetic ageing as a complementary rapid-ageing pathway. Given that significant investment is already underway, including a recently published epigenetic clock for Indian Ocean albacore (Chevrier et al., 2026) and ongoing CSIRO work on South Pacific albacore initiated under WCPFC Project 100c, the epigenetic ageing component of P129 has been carefully scoped to avoid duplication. In the interim, SPC is collecting high-quality tuna muscle tissue samples (preserved in liquid nitrogen), with paired otoliths, to ensure material is available should tuna epigenetic clock development be pursued under P129 in 2028/29. Together, these activities provide a strong foundation for the future implementation of routine age–length data collection programmes across the WCPFC area and demonstrate that Project P129 is progressing rapidly in delivering the original Phase 1 objectives and is adding considerable value through leveraging of other activities in this research area, and the new laboratory facilities and equipment available at SPC in Noumea.

RECOMMENDATIONS

SC22 is invited to:

1. Note the progress achieved under Phase 1 of Project P129, including the establishment of standardised ageing protocols for yellowfin and bigeye tuna, advances in LA-ICP-MS annual increment validation, preliminary rapid age-prediction modelling using otolith biometry, and the initiation of equivalent work for South Pacific albacore and skipjack tuna.

2. Prioritise the continuation of Project P129 into Phase 2, noting that the scientific foundations required for the development of an age–length data stream for tuna assessments are progressing.

3. Note the planned increase in albacore otolith sampling ahead of the 2027 stock assessment, and the importance of Member support for expanded sample collection across key ports and fishing sectors.

4. Endorse the proposed 2027 budget request of USD 90,000, comprising USD 60,000 for project activities and USD 30,000 for two microscopes with digital cameras and associated imaging equipment to increase otolith ageing capacity and support the development and validation of rapid ageing methods.

1. Background

Reliable age information is fundamental to tuna stock assessments because it contributes towards estimating growth, mortality, recruitment, productivity, and stock status. Despite the important role of age in age-structured population models, routine collections of conditional age-at-length (CAAL) data remain extremely limited for tropical tuna species throughout the western and central Pacific Ocean. Consequently, stock assessments for yellowfin, bigeye, albacore and skipjack tuna continue to rely heavily on static growth assumptions and univariate size-based proxies for age. Beyond improving the availability of CAAL data, there is also a need to generate representative age compositions from the large-scale otolith collections routinely obtained through regional sampling programmes. Achieving this using conventional sectioning and age interpretation for every otolith is logistically challenging, costly, and unlikely to be feasible at the scale required for routine stock assessment applications.

Age data limitations have been identified as a major source of uncertainty in the tuna stock assessments. Growth is known to vary spatially and temporally, and the application of static growth relationships may introduce bias, particularly when reconstructing population age structures from length-frequency data alone. Routine collection of CAAL data would enable stock assessments to estimate fishery- and region-specific age–length relationships, while rapid ageing approaches would allow representative age compositions to be generated from large otolith collections without requiring every otolith to be sectioned and individually read. Together, these advances provide a practical pathway for substantially increasing both the quantity and representativeness of age information available to WCPFC tuna stock assessments.

Project P129 was established to develop the scientific and operational foundations required to achieve scalable age estimation for key WCPFC-managed tuna species. The project aims to develop and validate surrogate rapid-ageing methodologies, including otolith biometric modelling and epigenetic ageing, and progress these approaches towards implementation within routine sampling programmes. A fundamental requirement of all surrogate ageing approaches is a robust, validated reference age dataset derived from conventionally aged otolith sections, which provides the calibration, training, and independent evaluation required to develop reliable age-prediction models.

Accordingly, the project follows a staged four-year approach, beginning with the refinement and validation of direct ageing methods before progressing to the development, validation, and implementation of rapid-ageing technologies.

This paper provides the first progress update for Project P129 and summarises achievements during the first six months against Phase 1 objectives.

2. Progress against Phase 1 objectives

The 2026 Phase 1 workplan for Project P129 comprised two primary objectives:

1. Accelerate the development of rapid-ageing methodologies for yellowfin and bigeye tuna using otolith morphometrics.
2. Assess the feasibility and sampling requirements for establishing a regional age–length sampling programme capable of supporting routine collection of representative age data for WCPFC stock assessments.

Substantial progress has been achieved during the first six months of the project, with activities advancing beyond the original Phase 1 workplan. In addition to progressing rapid-ageing model development, considerable effort has been directed towards establishing the scientific foundations required for their implementation, including the standardisation and validation of direct ageing methods, development of reference age datasets, and validation of age interpretation.

Progress has also commenced towards evaluating the sampling requirements and implementation pathways needed to support a regional age–length sampling programme. The following sections summarise progress against each of these activities.

3. Establishing reference age datasets

3.1. Standardisation of ageing methods

The development of reliable and rapid age prediction methods depends on robust age-based reference datasets that provide the calibration and training data required to develop epigenetic ageing and multivariate ensemble age-prediction models. Accordingly, considerable effort has focussed on revising and standardising direct ageing methodologies from otolith sections for tropical tunas. Commonly, annual age estimates have been derived from interpretations of annual growth zones in transverse sections of otoliths (Williams et al. 2013). However, oblique (sometimes referred to as longitudinal) sections have been shown to provide more precise daily age estimates because they provide a longer reading plane and wider increment spacing, resulting in greater growth-zone clarity than transverse sections (Williams et al. 2013). Previous studies have also demonstrated the utility of oblique sectioning methods for annual age estimation. Chen et al. (2012) applied oblique sections to estimate annual age in albacore tuna, while Shih et al. (2014) did so for yellowfin tuna, with both studies reporting that annual growth zones were easier to interpret from oblique sections.

To evaluate the suitability of this approach for tropical tuna ageing, annual growth zones were compared between oblique and transverse sections of yellowfin and bigeye tuna. Comparisons of oblique and transverse sections showed that the broader ventral region exposed by the oblique section provided a wider reading plane, improving the clarity and interpretability of annual growth zones. This finding was consistent with those reported by Chen et al. (2012) and Shih et al. (2014). Consequently, oblique sections were adopted as the standard preparation method for subsequent ageing (Fig. 1). Because historical age estimates for yellowfin and bigeye tuna had been derived from transverse sections (Farley et al. 2020), it was necessary to quantify the precision and comparability of the two preparation methods. To support method standardisation, a reference collection of obliquely sectioned otoliths was established for both species. Ageing precision was then assessed through independent multi-reader comparisons between experienced readers from Fish Ageing Services, who had aged the historical transverse sectioned samples, and SPC. In addition, a subset of the second otolith from yellowfin (n = 68) and bigeye tuna (n = 73) that had previously been aged using transverse sections were sectioned obliquely. Visual comparisons and chemical interpretations of annual growth zones, using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) analyses, were then performed to validate age estimates (see Section 3.2).

Precision in independent opaque zone counts among readers (n = 3) for the reference sets of obliquely sectioned yellowfin and bigeye tuna were assessed separately using pairwise comparisons of the index of average percent error (IAPE) relative to the generally accepted threshold (i.e., IAPE < 5.5%; Campana 2001). Dissimilarities in opaque zone counts among readers were visualised using a two-dimensional metric multi-dimensional scaling (mMDS) ordination, in which distances and directions of separation among data points represented relative distances in age estimates. An ellipse with a radius corresponding to an IAPE of 5.5% from the centroid was overlaid to facilitate interpretations (see Wakefield et al. 2017b; Wakefield et al. 2025; Wakefield et al. 2026).

The mMDS ordinations were considered an adequate representation of the underlying dissimilarities based on a strong 1:1 linear relationship in the Shepard diagram (i.e., between pairwise Euclidean dissimilarities and ordination distances) and a stress coefficient < 0.25 (see Clarke et al. 2014). Opaque zone interpretations from sectioned otoliths by three readers achieved acceptable inter-reader precision, with the distribution of data points falling within the ellipse (IAPE < 5.5% from the centroid) for both yellowfin and bigeye tuna (Figure 2). Pairwise comparisons of opaque zone counts among readers were less than 5.5% IAPE between readers 1 and 3 for yellowfin and readers 1 and 2 for bigeye tuna (Figure 2), and only slightly exceeded 5.5% for the remaining reader comparisons. These results demonstrate that annual growth zones can be consistently interpreted in oblique otolith sections and that acceptable levels of inter-reader precision can be achieved.

The opaque zone counts derived from oblique-sectioned otoliths were subsequently assigned decimal ages using the "*Jesstimation*" fractional ageing approach developed by CSIRO (Farley et al. 2025). Because this fractional ageing framework relies on calibration with daily-aged fish, a reference dataset was established using young-of-the-year (0+) yellowfin and bigeye tuna from Williams et al. (2013), for which daily ages had been determined from oblique otolith sections. The distance from the primordium to the otolith edge was measured for each 0+ fish to characterise otolith growth relative to known daily age and provide the calibration required for fractional age estimation. Following calibration, the distance from the primordium to the outer margin of each opaque zone was measured for all oblique-sectioned otoliths. These measurements were subsequently incorporated into the *Jesstimation* analysis to convert annual increment counts into decimal age estimates. Applying a common calibration derived from daily-aged fish ensured that decimal age estimates were standardised across the contemporary oblique-sectioned otoliths while remaining directly comparable with the historical age estimates derived from transverse otolith sections.

The consistency of the age-length relationships between transverse- and oblique-sectioned otoliths demonstrated that the two ageing approaches produced compatible age estimates (Figure 3). Considering the similar distributions in the age-length relationship for these datasets, otolith biometry from both sectioning methods were combined into a single standardised reference age dataset, which underpinned the subsequent development of surrogate age-prediction methods.

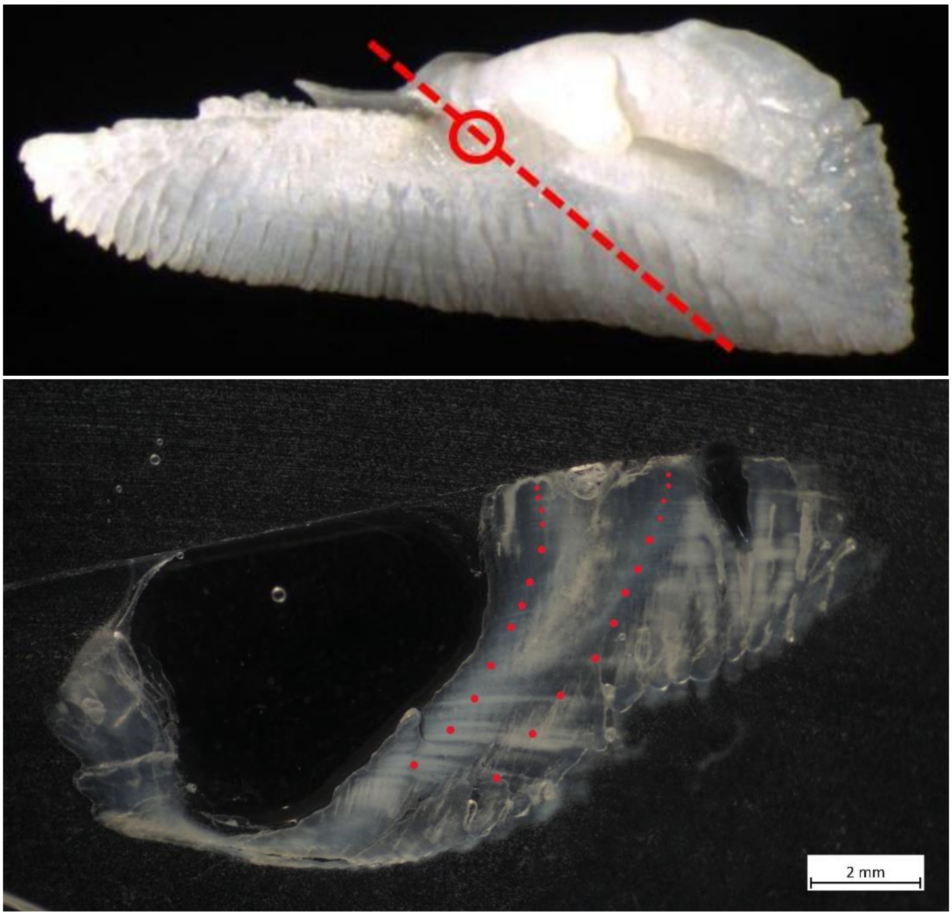

Figure 1. Whole (above) and oblique section (below, ~280 µm thick under reflected light) of the sagittal otolith of a male yellowfin tuna (1510 mm fork length) with 12 opaque zones (red circles) visible along two reading axes. Note the oblique section orientation (dashed red line) through the primordium (red circle) has also been denoted on the whole otolith image (above).

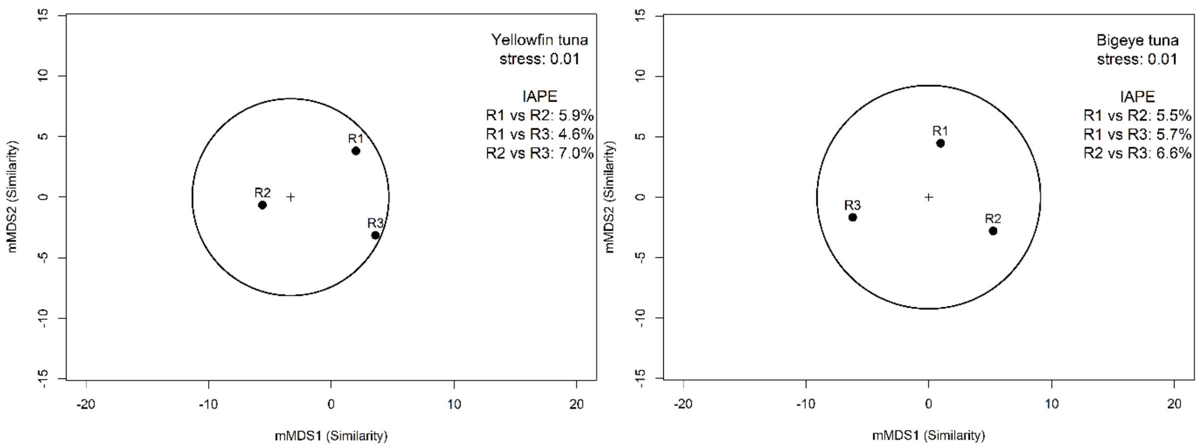

Figure 2. Metric multi-dimensional scaling (mMDS) ordination derived from independent opaque zone counts in oblique sections of otoliths by three experienced age readers (R1-R3) from reference sets for yellowfin (left, n = 78) and bigeye tuna (right, n = 93). The distance from the centroid (+) to the ellipse represents the generally accepted index of average percent error (IAPE of 5.5 %).

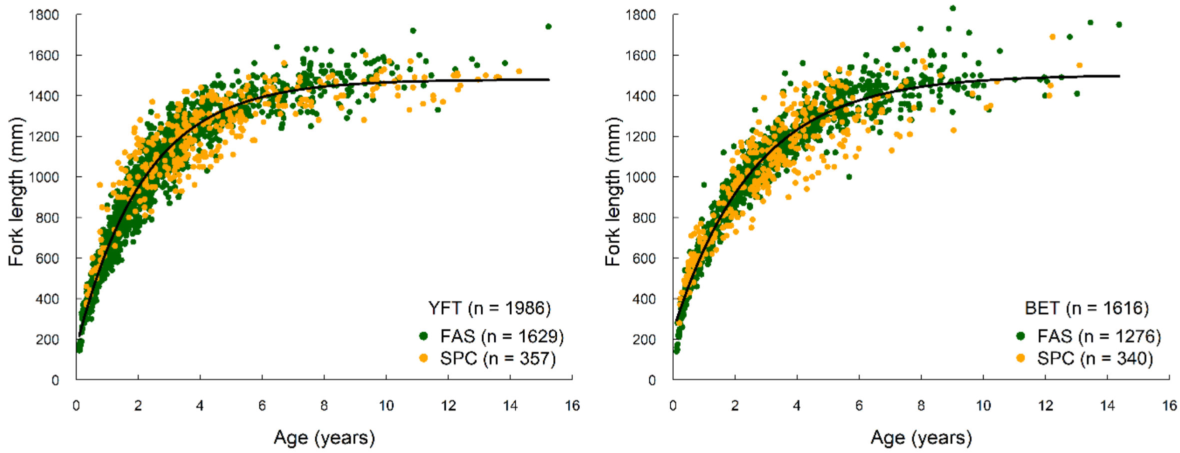

Figure 3. Length-at-age data for yellowfin tuna (YFT, left) and bigeye tuna (BET, right) estimated from transverse otolith sections by Farley et al. (2020) (FAS; green circles) and from oblique otolith sections by SPC (orange circles). The solid line represents the von Bertalanffy growth curve fitted to the combined dataset.

3.2. VALIDATION OF INCREMENT PERIODICITY USING LA-ICP-MS

Validating interpretations of annual opaque and translucent growth zone periodicity in sectioned otoliths remains a critical requirement for robust age estimates of tropical tunas (e.g., Farley et al. 2013; Andrews et al. 2024; Fraile et al. 2024). To provide an independent assessment of annual increment deposition, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) analyses have been completed on 391 otolith sections comprising 164 yellowfin tuna, 153 bigeye tuna, and 74 skipjack tuna.

Although analyses are ongoing, the objective of this work is to determine patterns of periodic and cyclical variation in otolith elemental composition and if they correspond with annual increment formation. Specifically, implementing primordium-to-edge laser ablation transects can reconstruct lifelong cyclical variation in elemental signatures linked to physiological processes that underpin growth-zone formation during otolith accretion (Heimbrand et al. 2020; Hüssy et al. 2021). Aligning these chemical signatures with visually interpreted growth zones provides an independent line of evidence supporting age interpretation in species with difficult-to-interpret otoliths.

To achieve this, elemental ratios associated with growth in marine fishes (^{24}Mg, ^{88}Sr, ^{44}Ca) were quantified using an Agilent 7900x ICP-MS coupled to a RESOlution LR 193 nm excimer laser ablation system. Laser ablation transects extended from the primordium to the outer margin of each otolith section to characterise elemental variation across the entire life history of each fish. Following analysis, the distance along the laser ablation scar to the outer margin of each opaque zone (i.e., annual age increment) was measured in ObjectJ (ImageJ plugin) by two experienced readers, enabling direct comparisons between chemical periodicity and age estimates.

Preliminary observations indicate consistent cyclical variation in elemental ratios associated with visually interpreted annual growth structures (Figures 4-6). Notably, while elemental ratios that exhibited the strongest periodic signal linked to age interpretation differed among species, initial findings demonstrate considerable potential for applying LA-ICP-MS as an age validation tool for tropical tunas.

A particularly valuable component of this work is the inclusion of paired datasets from fish previously aged using traditional methodologies. Specifically, 68 yellowfin tuna and 73 bigeye tuna analysed by

LA-ICP-MS were sister otoliths from individuals with historical age estimates derived from transverse sections (Farley et al. 2020). These paired datasets provide a unique opportunity to evaluate consistency among preparation methods and further quantify ageing precision (see Section 3.1).

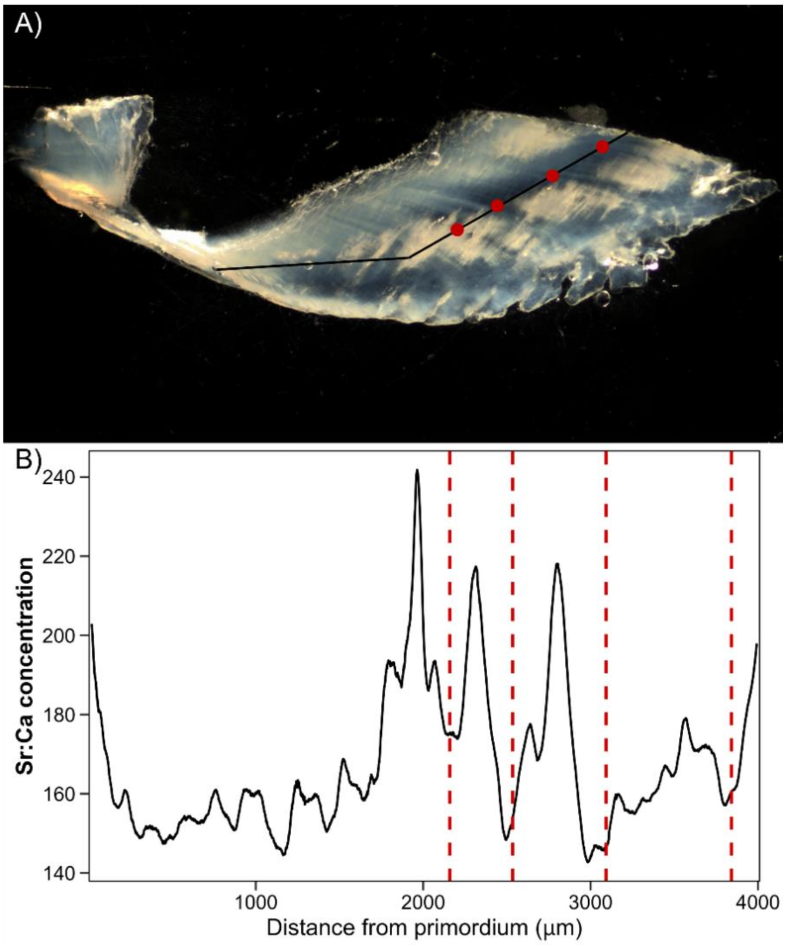

Figure 4. (A) Obliquely sectioned yellowfin tuna otolith imaged under reflected light at 2x magnification using a Zeiss Stemi 508 microscope coupled with a Zeiss Axiocam 208 colour camera. The black line indicates the laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) transect, while red dots denote the positions of visually interpreted annual opaque zones measured in ObjectJ (ImageJ). Red dots were positioned at the delineation of each opaque zone to represent the completion of each annual increment (n = 4 years). (B) Corresponding Sr:Ca chemical signature profile generated by LA-ICP-MS along the transect shown in panel A. The black line represents variation in the Sr:Ca elemental ratio from the primordium to the outer margin of the otolith, and vertical dashed red lines indicate the interpreted opaque zone positions used to assign age estimates in panel A.

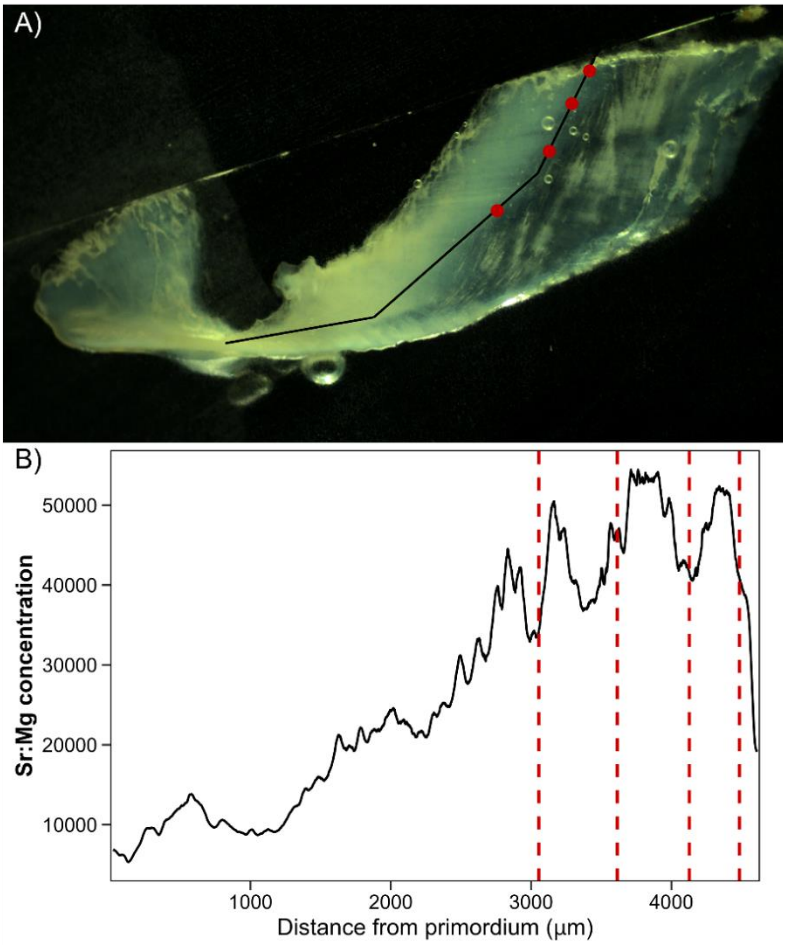

Figure 5. (A) Obliquely sectioned bigeye tuna otolith imaged under reflected light at 2x magnification using a Zeiss Stemi 508 microscope coupled with a Zeiss Axiocam 208 colour camera. The black line indicates the laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) transect, while red dots denote the positions of visually interpreted annual opaque zones measured in ObjectJ (ImageJ). Red dots were positioned at the delineation of each opaque growth-zone to represent the completion of each annual increment (n = 3 years). (B) Corresponding Sr:Mg chemical signature profile generated by LA-ICP-MS along the transect shown in panel A. The black line represents variation in the Sr:Mg elemental ratio from the primordium to the outer margin of the otolith, and vertical dashed red lines indicate the interpreted opaque zone positions used to assign age estimates in panel A.

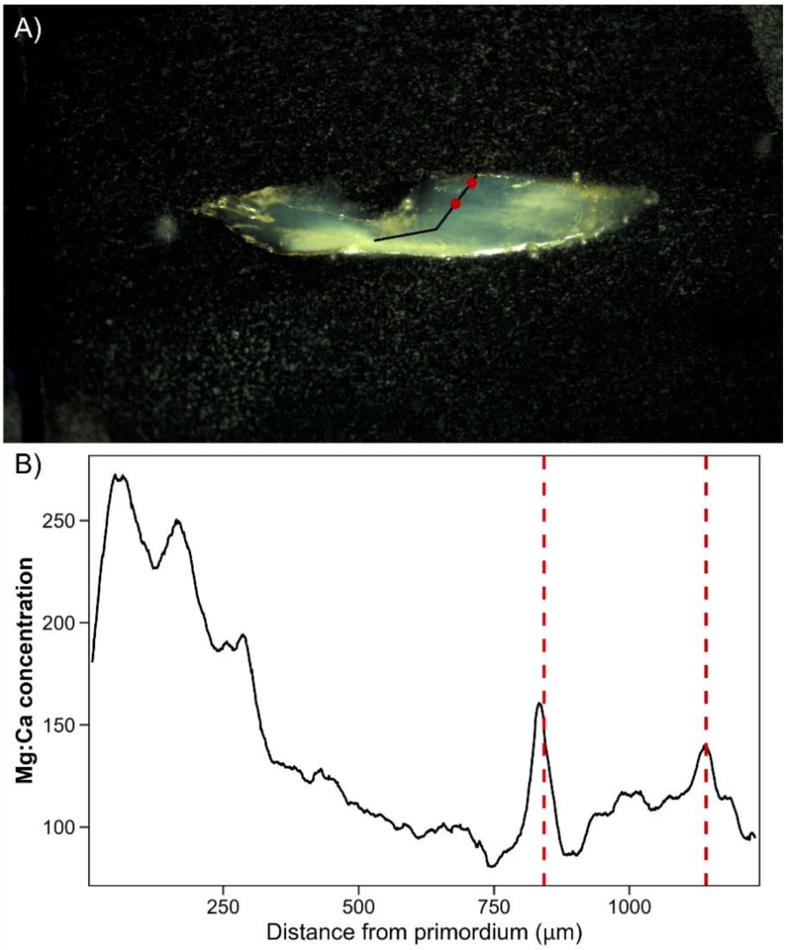

Figure 6. (A) Obliquely sectioned skipjack tuna otolith imaged under reflected light at 2x magnification using a Zeiss Stemi 508 microscope coupled with a Zeiss Axiocam 208 colour camera. The black line indicates the laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) transect, while red dots denote the positions of visually interpreted annual opaque zones measured in ObjectJ (ImageJ). Red dots were positioned at the delineation of each opaque growth-zone to represent the completion of each annual increment (n = 2 years). (B) Corresponding Mg:Ca chemical signature profile generated by LA-ICP-MS along the transect shown in panel A. The black line represents variation in the Mg:Ca elemental ratio from the primordium to the outer margin of the otolith, and vertical dashed red lines indicate the interpreted opaque zone positions used to assign age estimates in panel A.

6. Rapid age prediction using otolith biometrics

A key objective of Project P129 is the development of rapid ageing methodologies capable of generating age information at scales suitable for representative fisheries monitoring programs spanning the broad western and central Pacific region. Following standardisation of historical and contemporary age datasets, otolith biometric measurements have been compiled from both archived and recently aged specimens. These data provide the foundation for development of predictive age models.

Model development during Phase 1 is focussed on yellowfin and bigeye tuna and employs a multivariate gradient boosting machine (GBM) ensemble approach (see Wakefield et al. 2026). Predictor variables include otolith biometric measurements, fish length, sex, and spatial information (i.e. latitude and longitude of capture location). Otolith morphometrics for the sister otoliths of the yellowfin and bigeye tuna aged by Farley et al. (2020) and those more recently aged using oblique sections, were collected following the methods outlined by Williams et al. (2015) and Wakefield et al. (2026). This involved using calibrated digital callipers to measure the basic dimensions of each otolith to the nearest 0.01 mm, including (i) length of the otolith (from rostrum to post-rostrum), (ii) width at the widest point adjacent to the primordium, and (iii) thickness across the transverse plain over the primordium. Each otolith was weighed to the nearest 0.0001 g using a calibrated balance with draft shields. Otolith surface area and perimeter were derived from images of the distal surface of whole otoliths using shape analysis following the procedures described by Libungan and Palsson (using the ShapeR package; 2015).

GBM models were tuned using 5-fold cross-validation and evaluated using a summary grid implemented in the caret package in R (Kuhn 2008). Optimal model parameters were selected based on minimisation of root mean squared error (RMSE), and final models were fitted using a Gaussian distribution with the gbm package in R (Ridgeway and Ridgeway 2004). Overfitting was assessed by comparing training and cross-validation error, and the optimal number of boosting iterations was defined as the point at which validation error was minimised. The relative influence of otolith, biological and location variables on age prediction was quantified, and model performance was evaluated using index of average percent error (IAPE) and age-bias plots with LOESS curves fitted to residuals.

Otolith biometric data were measured for a total of 670 yellowfin (n = 397 and 273, from Farley et al. (2020) and recent oblique sections, respectively) and 416 bigeye tuna (n = 138 and 278, from Farley et al. (2020) and recent oblique sections, respectively), ranging in age from 0-14 and 0-12 years, respectively. Univariate relationships between all otolith morphometric measurements and age did not differ significantly between females and males for either yellowfin (Figure 7) or bigeye tuna (Figure 8). Relationships between most otolith dimensions (i.e., otolith length, width and perimeter) and age were curvilinear, approaching an asymptote from approximately four years of age (Figures 7 & 8). In contrast, relationships between otolith weight, area and thickness with age were generally linear across the observed age range, indicating continuous accretion of the medial (i.e., dorsal and ventral to the sulcus) and distal surfaces at a relatively constant rate throughout the lifespan of both species. Consistent with this pattern, otolith weight was the most influential predictor of age in the GBM models, followed by fish length, for both species (Figure 9).

Considering age compositions of yellowfin and bigeye tuna are analysed at quarterly age classes within the stock assessment models, age prediction performance may need to be evaluated at finer decimal age resolutions. In this study, age predictions are presented to the nearest half-year age class. Prediction precision at this resolution was similar for the two species, with IAPE values of 7.6%

for yellowfin and 9.4% for bigeye tuna. Age-bias plots exhibited similar trends for both models up to six years of age, characterised by strong linear relationships between observed and predicted ages (R^2 = 0.92 and 0.89 for yellowfin and bigeye tuna models, respectively). However, predicted ages for bigeye tuna showed an increasing tendency to be underestimated beyond approximately six years of age, as indicated by the LOESS curve fitted to the residuals (Figure 10). This bias most likely reflects the markedly lower representation of older individuals in the training dataset (Figure 10). It is also consistent with the relatively low influence of otolith thickness in the models (Figure 9), despite previous work showing that this measurement becomes increasingly more influential for predicting ages of older fish (see Wakefield et al. 2026).

These preliminary results demonstrate that simple otolith morphometric measurements (i.e., length, width, thickness and weight), together with fish length and spatial coordinates, can predict age with reasonable precision for yellowfin and bigeye tuna. Using the current reference dataset, model performance is strongest for fish up to six years of age, with precision at older ages likely to improve as additional older individuals are incorporated into the training data (see Wakefield et al. 2026). This approach provides a rapid and cost-effective alternative to routine otolith sectioning and age determination and has the potential to support the standardisation of ageing protocols across laboratories through the application of consistent, reproducible age-predictions.

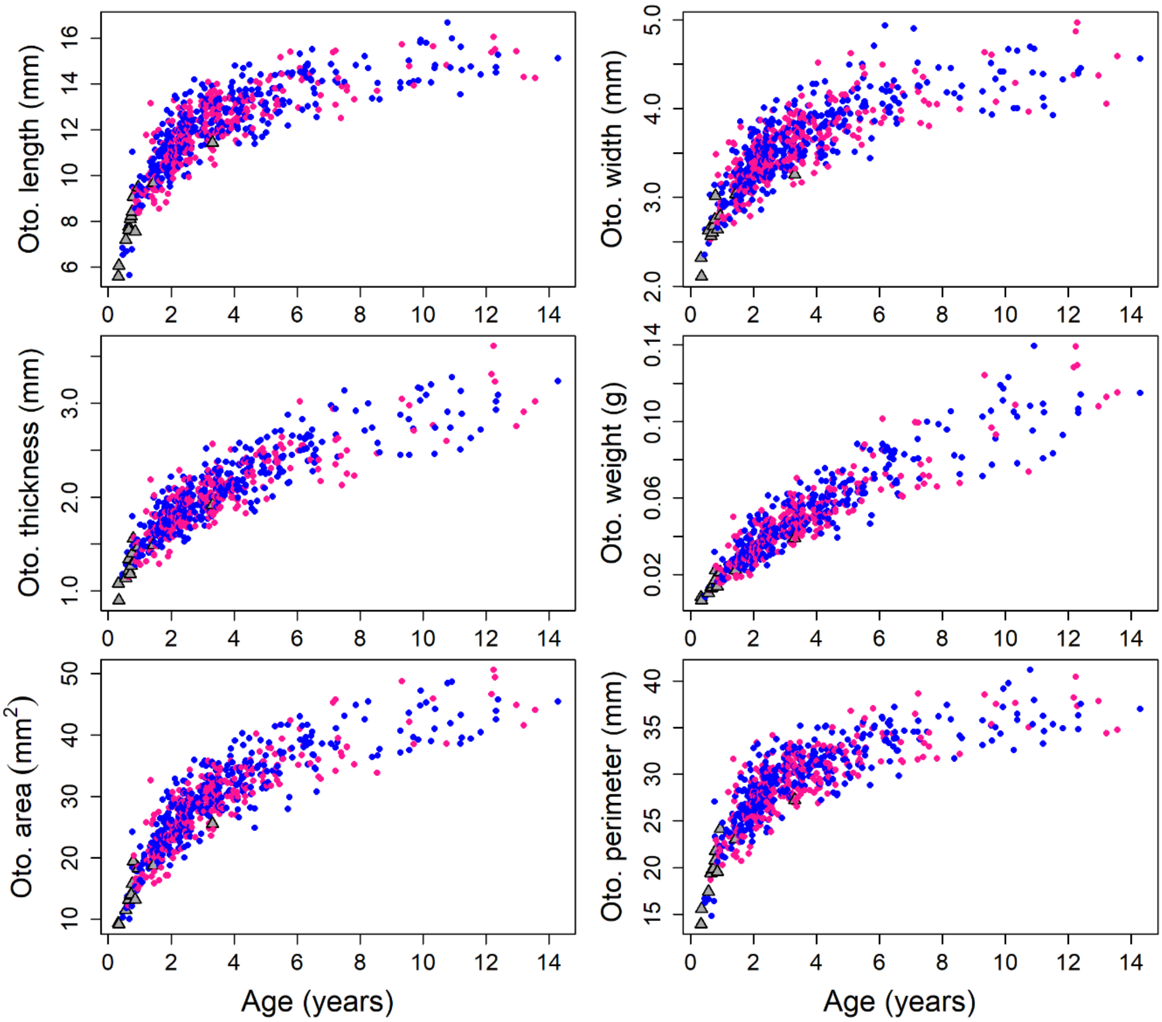

Figure 7. Univariate relationships between otolith biometry (i.e., length, width, thickness, weight, area and perimeter) and age (0.3-14.3 years derived from sectioned otoliths) for female (pink circles, n = 275), male (blue circles, n = 370) and undetermined sex (grey triangles, n = 25) of yellowfin tuna from the Pacific Ocean. There were no significant differences between sexes for all biometric relationships with age.

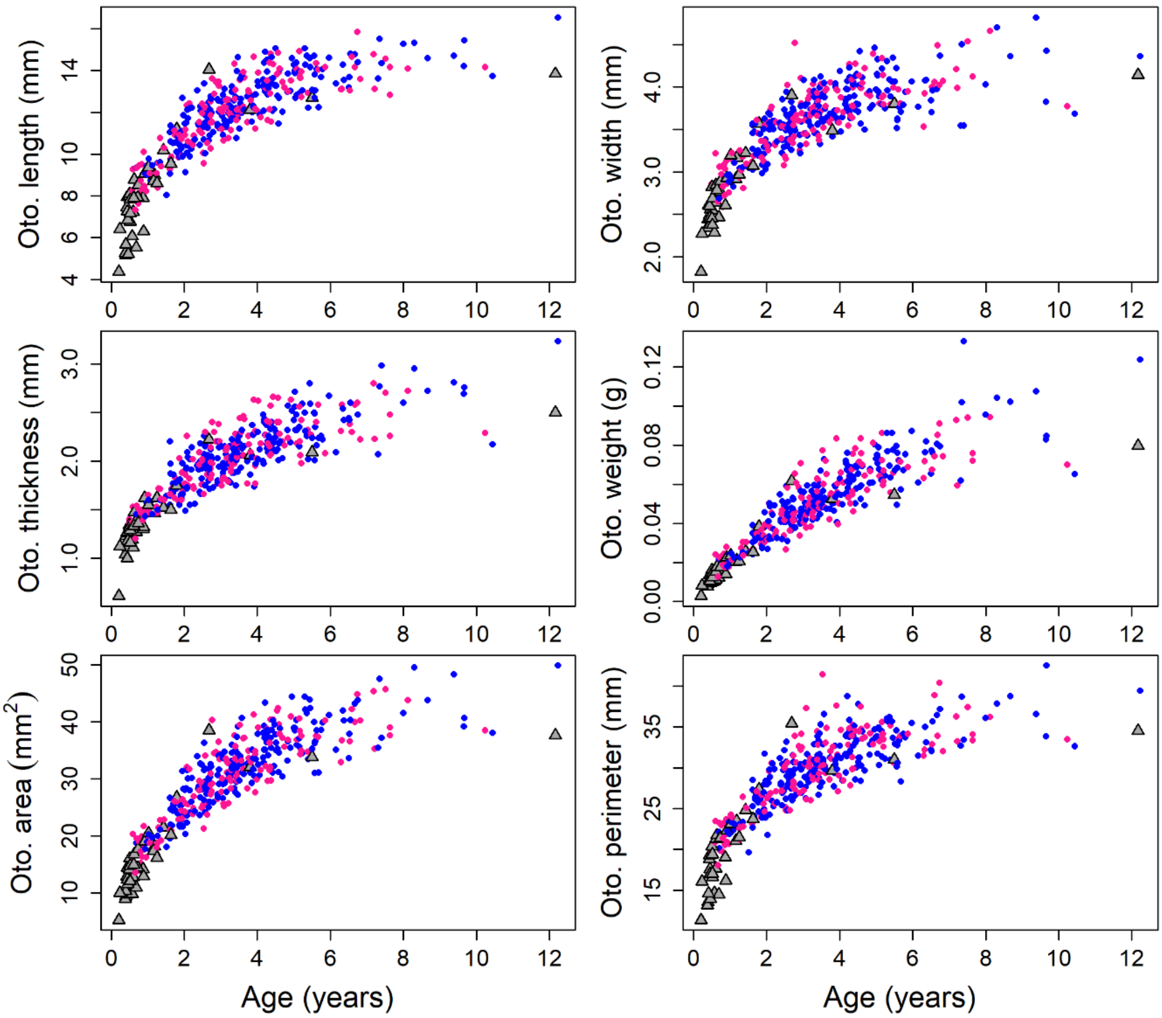

Figure 8. Univariate relationships between otolith biometry (i.e., length, width, thickness, weight, area and perimeter) and age (0.2-12.3 years derived from sectioned otoliths) for female (pink circles, n = 143), male (blue circles, n = 213) and undetermined sex (grey triangles, n = 60) of bigeye tuna from the Pacific Ocean. There were no significant differences between sexes for all biometric relationships with age.

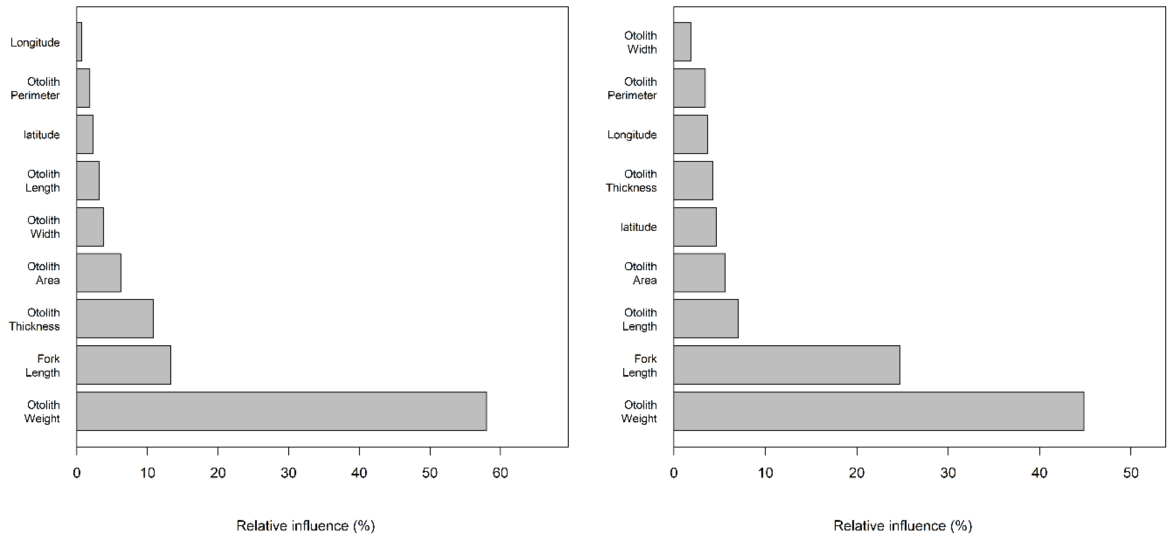

Figure 9. Relative influence of each otolith biometric, biological and spatial variable for predicting ages (rounded to the nearest half yearly age class) using a Gradient Boosting Machine model for yellowfin (left) and bigeye tuna (right).

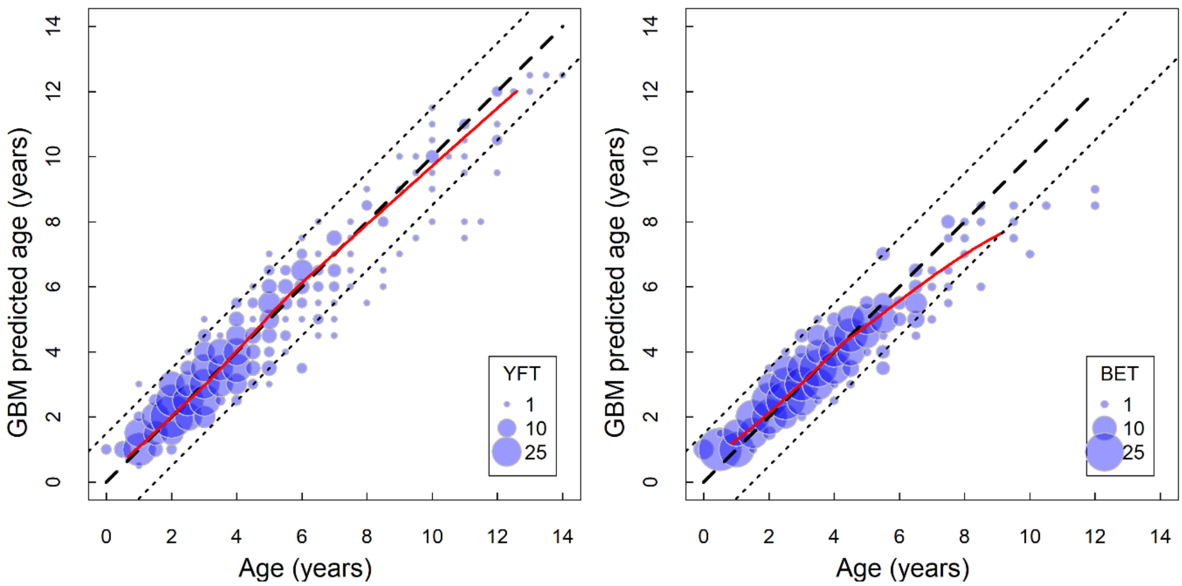

Figure 10. Age-bias plots comparing ages (rounded to the nearest half year) derived from otolith sections and those predicted using a Gradient Boosting Machine (GBM) model for yellowfin (left, YFT) and bigeye tuna (right, BET). Size of blue circles relative to sample sizes, see legend; equivalent ages, thick black dashed line; ages ±1 year, thin black dashed lines; residuals (loess curve), red line.

7. Epigenetic ageing

Epigenetic ageing has the potential to substantially increase the availability of age data for fisheries assessments by enabling age prediction from tissue samples, which are considerably easier to collect through routine observer and port-sampling programmes than otoliths. This approach could significantly reduce the logistical complexity and reliance on specialist expertise associated with collecting, processing, and interpreting otoliths, while facilitating age-length data collection at scales that are currently impractical using traditional ageing methods. Although the current cost of epigenetic age estimation remains higher than conventional otolith-based ageing, ongoing advances in sequencing technologies, analytical workflows, and high-throughput processing are expected to substantially reduce these costs over time.

Significant investment in tuna-specific epigenetic ageing is already being undertaken through other research initiatives. Accordingly, any future contribution by WCPFC in this area should be carefully scoped to complement, rather than duplicate, these existing efforts. A recent study by Chevrier et al. (2026) produced a *de novo* reference genome for Indian Ocean albacore tuna and constructed an epigenetic clock using ultra-conserved elements (UCEs) from muscle tissue samples, achieving acceptable ageing precision across an age range of 0–18 years. Concurrently, as reported to SC21 (SPC-OFP and CSIRO, 2025), CSIRO is developing epigenetic ageing methods for South Pacific albacore and southern bluefin tuna, in collaboration with Diversity Arrays Technology (DArT), to improve analytical workflows and develop high-throughput approaches suitable for routine fisheries applications, including close-kin mark–recapture (CKMR). The goal of that work is to develop a single assay that can be applied across several tuna species, including the tropical tunas, providing a scalable and commercially viable approach for fisheries applications.

Given these active programmes, the application of epigenetic ageing to Pacific tuna species under Project 129 is contingent on the outcomes of these ongoing studies. In the interim, SPC is concurrently collecting high-quality tuna muscle tissue (preserved in liquid nitrogen) and paired otolith samples, to ensure appropriate reference material is available should development of tuna-specific epigenetic clocks be warranted in the future. The indicative cost of developing an epigenetic ageing approach is approximately USD 75,000 per species. While this investment is not currently required under Project 129, it could be considered for inclusion in future project planning for 2028/29, subject to the progress and outcomes of the existing programmes.

Together, the development of otolith biometric age-prediction models, strategic collection of high-quality tissue samples, and continued monitoring of international advances in epigenetic ageing of tunas provide complementary pathways for reducing project risk. This staged approach maximises future opportunities to establish operational rapid-ageing methodologies that support scalable age–length data collection for Pacific tuna stock assessments.

8. Future Activities

Consistent with the original four-phase implementation plan, activities over the remainder of Phase 1 (2026) will continue to focus on advancing rapid ageing methods and assessing the feasibility of establishing a regional age-length sampling program. Priority activities include:

• Completion of LA-ICP-MS analyses and formal statistical validation of increment periodicity for yellowfin and bigeye tuna, confirming the reliability of reference age datasets for surrogate model calibration;

• Finalisation of a validated direct ageing protocol for skipjack tuna, drawing on advances in oblique sectioning and LA-ICP-MS analyses achieved during Phase 1, and providing the reference age dataset required for subsequent rapid ageing model development using otolith biometrics;

• Finalisation of ageing precision assessments between oblique and transverse otolith sections and ageing error matrices for yellowfin and bigeye tuna;

• Expansion of older age classes currently underrepresented in reference datasets and continued refinement of GBM age-prediction models for yellowfin and bigeye tuna;

• Development of GBM age-prediction models for South Pacific albacore using otolith biometrics and fish length, supported by increased otolith sampling ahead of the 2027 South Pacific albacore stock assessment;

• Continued evaluation of epigenetic ageing technologies, with future skipjack epigenetic clock development contingent on finalisation of validated direct ageing protocols; and

• Continued consultation with WCPFC members, Distant Water Fishing Nations, Regional Observer Programme coordinators, and partner organisations to assess the logistical requirements, sampling capacity, and long-term feasibility of implementing a regional age-length sampling program at an operational scale.

Successful completion of these activities will position the project to transition into Phase 2 (2027), where emphasis will shift towards peer-reviewed validation of rapid ageing methods, development of regional sampling protocols and training materials, establishment of sample collection and transport logistics, and evaluation of the costs associated with routine implementation of a regional age-length sampling program. Subject to successful completion of these milestones, Phase 3 (2028) will focus on trial implementation, followed by refinement and full implementation during Phase 4 (2029).

To support these activities, we would like to request additional funding for two microscopes with digital cameras and associated imaging equipment. This will increase the otolith ageing capacity required for this project and accelerate the development and validation of rapid ageing methods.

9. Conclusions

Project P129 has made substantial progress during the first six months of Phase 1 towards establishing the scientific foundations required for a scalable regional age-length data stream to support WCPFC tuna stock assessments. A key outcome to date has been the development of robust reference age datasets derived from directly aged otolith sections. This has appropriately prioritised the standardisation of ageing protocols, quantification of ageing precision, and validation of annual increment periodicity using LA-ICP-MS before surrogate age-prediction methods are fully developed.

Validated reference datasets have now been established for yellowfin and bigeye tuna, with preliminary GBM age-prediction models demonstrating encouraging levels of precision. In parallel, advances in oblique otolith sectioning and LA-ICP-MS analyses have provided, for the first time, a credible pathway for direct ageing of skipjack tuna, establishing the foundation required for future development of both otolith biometric and epigenetic age-prediction methods. Development of rapid age-prediction models has also commenced for South Pacific albacore through expanded otolith sampling ahead of the 2027 stock assessment.

In parallel with the continued development of rapid ageing methods, the project will now commence activities to assess the feasibility of implementing a regional age-length sampling programme, as originally proposed for Phase 1. Initial efforts will focus on expanding otolith collections, particularly for South Pacific albacore ahead of the 2027 stock assessment, while engaging with WCPFC members, Distant Water Fishing Nations, Regional Observer Programme coordinators, and other partner organisations to evaluate sampling capacity, logistical requirements, and the long-term feasibility of a routine regional sampling programme. The project will also investigate establishing a Pacific regional otolith reference library, linking archived otoliths with biological data, otolith shape biometric data, annual growth increment measurements, and age estimates. This resource would support, among other things, the development of rapid ageing methods while promoting consistent age estimation across the Pacific.

The transformative potential of these methods lies not only in enabling the collection of conditional age-at-length data, but also in their capacity to derive representative age compositions from large otolith collections without requiring every otolith to be sectioned and individually aged. This represents a substantial step towards improving the quantity, consistency and spatial coverage of age data available for stock assessments of yellowfin, bigeye, South Pacific albacore and skipjack tuna.

Overall, progress achieved during the first six months indicates that Project P129 remains on schedule with the original phased implementation plan. Completion of the remaining Phase 1 activities will provide the validated ageing methods, reference datasets, and implementation framework needed to transition into Phase 2, where the focus will broaden from method development to regional capacity building, sampling logistics, and operational implementation.

10. Acknowledgements

This work is primarily supported by funding from WCPFC Project 129: Building and age-length data stream for tuna assessments, with additional funding from the EU PEUMP 2 and GCF regional tuna projects.

We acknowledge the Pacific Marine Specimen Bank for providing the samples used in this study (SC22-RP-P35b-01), including the fisheries observers and research staff who collect and maintain samples. We also thank the WCPFC for granting access to the PMSB. The authors acknowledge the instruments and expertise of Microscopy Australia (ROR: 042mm0k03) at Adelaide Microscopy, Adelaide University enabled by NCRIS, university, and state government support. A special thanks to Paul Olin and Sarah Gilbert for all the help.

Declaration of generative AI and AI-assisted technologies in this SC paper preparation process: Generative AI tools (ChatGPT) were used in a minor capacity to assist with drafting and editing limited portions of the text in this document. All conclusions are those of the authors, who reviewed and verified all AI-assisted output. The methodology, model implementation, and the scientific conclusions are the responsibility of the authors.

11. References

Andrews, A.H., Eveson, J.P., Welte, C., Okamoto, K., Satoh, K., Krusic-Golub, K., Lougheed, B.C., Macdonald, J.I., Roupsard, F. and Farley, J.H. (2024) Age validation of yellowfin and bigeye tuna using post-peak bomb radiocarbon dating confirms long lifespans in the western and central Pacific Ocean. *ICES Journal of Marine Science*, 81(6), pp.1137-1149.

Campana, S.E. and Thorrold, S.R. (2001). Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can. J. Fish. Aquat. Sci. 58, 30–38.

Chen, K.S., Shimose, T., Tanabe, T., Chen, C.Y. and Hsu, C.C. (2012). Age and growth of albacore *Thunnus alalunga* in the North Pacific Ocean. *Journal of fish biology*, 80(6), pp.2328-2344.

Chevrier, T., Bonhommeau, S., Thompson, M., Farley, J., Del Vecchio, G., Nieblas, A.-E., Cowart, D.A., Imbert Nguyễn, J., Bernard, S., Guiguen, Y., Cabau, C., Klopp, C., Zoller, J.A., Horvath, S., Brooke, R., Lu, A.T., Pellegrini, M. and Chanut, J. (2026). Searching for shared epigenetic clocks: evaluating ultra-conserved markers in a de novo genome assembly of the albacore tuna. GeroScience. https://doi.org/10.1007/s11357-026-02192-0

Clarke, K.R., Gorley, R.N., Somerfield, P.J. and Warwick, R.M. (2014) Change in marine communities: an approach to statistical analysis and interpretation, 3rd edition. PRIMER-E.

Farley, J.H., Williams, A.J., Clear, N.P., Davies, C.R. and Nicol, S.J. (2013) Age estimation and validation for South Pacific albacore *Thunnus alalunga*. *Journal of fish biology*, 82(5), pp.1523-1544.

Farley, J., Krusic-Golub, K., Eveson, P., Clear, N., Roupsard, F., Sanchez, C., Nicol, S. and Hampton, J. (2020) Age and growth of yellowfin and bigeye tuna in the western and central Pacific Ocean from otoliths. Working Paper WCPFC-SC16-2020/SA-WP-02, Scientific Committee Sixteenth Regular Session, Electronic Meeting, 11-20 August 2020.

Farley, J.H., Eveson, J.P., Krusic-Golub, K., Clear, N.P., Sanchez, C. and Nicol, S.J. (2025). Jesstimation: A novel approach for estimating more accurate fish ages from otolith zone counts and measurements. *Fisheries Research*, 288, p.107463.

Fraile, I., Luque, P.L., Campana, S.E., Farley, J.H., Krusic-Golub, K., Clear, N., Eveson, J.P., Artetxe-Arrate, I., Zudaire, I., Murua, H. and Merino, G. (2024) Age validation of yellowfin tuna *Thunnus albacares* in the Indian Ocean using post-peak bomb radiocarbon chronologies. *Marine Ecology Progress Series*, 734, pp.91-104.

Heimbrand, Y., Limburg, K.E., Hüssy, K., Casini, M., Sjöberg, R., Palmén Bratt, A.M., Levinsky, S.E., Karpushevskaia, A., Radtke, K. and Öhlund, J. (2020). Seeking the true time: Exploring otolith chemistry as an age-determination tool. *Journal of fish biology*, 97(2), pp. 552-565.

Hüssy, K., Limburg, K.E., De Pontual, H., Thomas, O.R., Cook, P.K., Heimbrand, Y., Blass, M. and Sturrock, A.M. (2021). Trace element patterns in otoliths: the role of biomineralization. *Reviews in Fisheries Science & Aquaculture*, 29(4), pp. 445-477.

Libungan, L.A. and Pálsson, S. (2015). ShapeR: an R package to study otolith shape variation among fish populations. *PLoS ONE* 10, e0121102

Ridgeway, G. and Ridgeway, M.G. (2004). The gbm package. R Foundation for Statistical Computing, Vienna, Austria 5.

Shih, C.L., Hsu, C.C. and Chen, C.Y. (2014). First attempt to age yellowfin tuna, *Thunnus albacares*, in the Indian Ocean, based on sectioned otoliths. *Fisheries Research*, 149, pp.19-23.

SPC-OFP and CSIRO (2025). Progress towards a close-kin mark-recapture application to South Pacific albacore (Project 100c). Working Paper WCPFC-SC21-2025/SA-WP-09, Scientific Committee Twenty-First Regular Session, Nuku'alofa, Tonga, 13–21 August 2025. https://meetings.wcpfc.int/node/26685

Wakefield, C.B., O'Malley, J.M., Williams, A.J., Taylor, B.M., Nichols, R.S., Halafihi, T., Humphreys Jr, R.L., Kaltavara, J., Nicol, S.J. and Newman, S.J. (2017b). Ageing bias and precision for deep-water snappers: evaluating nascent otolith preparation methods using novel multivariate comparisons among readers and growth parameter estimates. *ICES Journal of Marine Science*, 74(1), 193-203.

Wakefield, C.B., Ong, J.J., Williams, A.J., Taylor, B.M., Andrews, A.H., Burton, M., Nicol, S.J. and Newman, S.J. (2025). Sclerochronological age synchrony corroborates remarkable lifespan and protracted asymptotic growth for a deepwater snapper (*Pristipomoides zonatus*) in the Indian and Pacific Oceans. *Fisheries Research*, 291.

Wakefield, C.B., Loudon, L., Harvey, E.S., Nicol, S.J., Williams, A.J. and Newman, S.J. (2026). High precision in age predictions derived from multivariate ensemble analyses of otolith biometry for a long-lived deepwater snapper. *Fisheries Research*, 294, p.107656.

Williams, A.J., Leroy, B.M., Nicol, S.J., Farley, J.H., Clear, N.P., Krusic-Golub, K., and Davies, C.R. (2013). Comparison of daily- and annual- increment counts in otoliths of bigeye (*Thunnus obesus*), yellowfin (*T. albacares*), southern bluefin (*T. maccoyii*) and albacore (*T. alalunga*) tuna. *ICES Journal of Marine Science*, 70(7), pp. 1439–1450, https://doi.org/10.1093/icesjms/fst093

Williams, A.J., Newman, S.J., Wakefield, C.B., Bunel, M., Halafihi, T., Kaltavara, J., and Nicol, S.J. (2015). Evaluating the performance of otolith morphometrics in deriving age compositions and mortality rates for assessment of data-poor tropical fisheries. *ICES J. Mar. Sci.* 72, 2098–2109.